GEORGIA INSTITUTE OF TECHNOLOGY — OFFICE OF CONTRACT ADMINISTRATION

PROJECT ADMINISTRATION DATA SHEET

[x] ORIGINAL [ ] REVISION NO. _____

Project No. A-3055 DATE 9/18/81

Project Director: Mr. James C. Toler School/Lab ~~XXX~~ ECSL/BRD

Sponsor: Air Force Systems Command, Aeronautical Systems Div., Wright Patterson AFB

Type Agreement: SFRC Contract No. F33615-81-K-0620

Award Period: From 9/01/81 To ~~3/01/82~~ 2/1/83 (Performance) 6/1/82 (Reports)

Sponsor Amount: $116,726 (incremental funded at $30,000 thru 9/30/81) Contracted through:

Cost Sharing: N/A GTRI/~~GIT~~ ~~XXX~~

Title: Operational Evaluation of New 435-MHz RFR Facility

ADMINISTRATIVE DATA

OCA Contact Faith G. Costello

1) Sponsor Technical Contact:

James H. Merritt
Scientific Program Officer
USAFSAM/RZP
Brook AFB, TX 78235

PH: (512) 536-3582

2) Sponsor Admin/Contractual Matters:

Thomas A. Bryant
ONR RR
206 O'Keefe Bldg.
Georgia Institute of Technology
Atlanta, Georgia 30332

Defense Priority Rating: _____ Security Classification: Unclassified

RESTRICTIONS

See Attached SFRC Supplemental Information Sheet for Additional Requirements.

Travel: Foreign travel must have prior approval — Contact OCA in each case. Domestic travel requires sponsor approval where total will exceed greater of $500 or 125% of approved proposal budget category.

Equipment: Title vests with GIT, subject to a right of the Gov't to direct transfer of the title to the Gov't or a 3rd party within 12 months after completion of contract.

COMMENTS:

COPIES TO:

Administrative Coordinator
Research Property Management
Accounting
Procurement/EES Supply Services
Research Security Services
~~Reports Coordinator (OCA)~~
Legal Services (OCA)
Library
EES Public Relations (2)
Computer Input
Project File
Other _____

FORM OCA 4:781

GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION SHEET

SR 106 81

Date 5/18/83

Project Title: Operational Evaluation of New 435-MHz RFR Facility

Project No: A-3055

Project Director: James C. Toler

Sponsor: AFSC, ASD, Wright-Patterson AFB

Effective Termination Date: 2/1/83

Clearance of Accounting Charges: 2/1/83

Grant/Contract Closeout Actions Remaining:

- [x] Final Invoice and Closing Documents
- [ ] Final Fiscal Report
- [x] Final Report of Inventions
- [x] Govt. Property Inventory & Related Certificate
- [ ] Classified Material Certificate
- [ ] Other _____

Assigned to: ECSL/BRD (~~School~~/Laboratory)

COPIES TO:

Administrative Coordinator	Research Security Services	EES Public Relations (2)
Research Property Management	Reports Coordinator (OCA)	Computer Input
Accounting	Legal Services (OCA)	Project File
Procurement/EES Supply Services	Library	Other Toler

FORM OCA 10:781

A-3055

ENGINEERING EXPERIMENT STATION
GEORGIA INSTITUTE OF TECHNOLOGY • ATLANTA, GEORGIA 30332

October 20, 1981

Department of the Air Force
Air Force Systems Command
Aeronautical Systems Division/PMRNB
Wright-Patterson AFB, OH 45433

Attention: Mr. James H. Merritt, USAFSAM/RZP

Reference: Contract No. F33615-81-K-0620
Operational Evaluation of New 435-MHz RFR Facility
Georgia Tech Project A-3055

Subject: Performance and Cost Report No. 1
Reporting Period: 18 September 1981 through 30 September 1981

Gentlemen:

Performance and cost information for the referenced contract are presented below for the reporting period 18 September 1981 through 30 September 1981.

I. Performance Information

Although the initiation date for the contract is officially 1 September 1981, notification of the award was not received until 18 September 1981. This notification of award also indicated that a funding increment of $30,000 was authorized for the period 1 September 1981 through 30 September 1981. Efforts were begun immediately to prepare the subcontract with Emory University. This subcontract requires Emory University personnel, under the direction of Dr. V. Popovic, to perform the following tasks:

1. Train Biomedical Technologists in cannulation techniques and micro-assay techniques. (Reference Sec. II.1 of Proposal No. ET-BR-1118(R1)) The training will include selecting healthy rats for cannulation, implanting the cannulae, monitoring the post-surgery health status of the rats, obtaining blood samples using the cannulae, and using micro-samples of blood to perform assays of biological endpoints.

2. Provide 100 rats, 50 of which are cannulated, for use during the six-week simulated experiment to "shakedown" the 435-MHz radiation facility at Georgia Tech. (Reference Sec. II.4 of Proposal No. ET-BR-1118(R1)).

3. Participate in the six-week simulated experiment by drawing micro-samples of blood from the 50 cannulated rats and using these samples to assay the following biological endpoints: total white blood cell count, differential count, hematocrit ratio, corticosterone, ACTH, growth hormone, prolactin, catecholamines, and arterial blood pressure. (Reference Sec. II.4 of Proposal No. ET-BR-1118(R1)).

4. Provide written inputs for combination with Georgia Tech inputs to satisfy the attached Contract Data Requirements List (monthly Performance and Cost Reports, Draft Report, and Final Report). Monthly Performance and Cost Reports are due to the Air Force on the 10th of each month beginning 10 October 1981. The Draft and Final Reports are due to the Air Force on 1 April 1982 and 1 June 1982, respectively.

An initial funding increment of $27,208.19 was provided to Emory University for this work.

Specific plans and procedures were also initiated for dosimetry determinations using the circular, parallel-plate radiation facility. Previously, field characteristics were measured using probe antennas and a single tier, prototype version of the facility. Preliminary measurements were also made on the present facility, and expected values of field strength and uniformity were confirmed. These measurements did not involve either animals or phantom models, and in-house instrumentation required for convenient dosimetry determinations is still in the process of being assembled. Therefore, Mr. Howard Bassen of the Bureau of Radiological Health (BRH) was contacted to inquire about the use of E-field probes specially designed for dosimetry determinations. Mr. Bassen agreed to loan a three-dimension, open air probe and a one-dimension, implantable probe to the project. These probes will include the E-field sensor, interconnecting fiber optic lead, and electronic module for signal processing and display. Probe availability will be approximately 20 November 1981.

II. Cost Information

a. Costs incurred during this reporting period:

Personal Services	$1,621.06
Fringe Benefits	180.11
Overhead	990.64
Materials and Supplies	0.00
Equipment	0.00
Travel	0.00
Computer Time	0.00
Emory Subcontract	27,208.19
TOTAL	$30,000.00

b. Cumulative costs through this reporting period:

Personal Services	$1,621.06
Fringe Benefits	180.11
Overhead	990.65
Materials and Supplies	0.00
Equipment	0.00
Travel	0.00
Computer Time	0.00
Emory Subcontract	27,208.19
TOTAL	$30,000.00

c. Amount vouchered to ACO: $2,791.81

d. Estimated funds necessary to complete project: $86,700.

Respectfully submitted,

J. C. Toler
Project Director

APPROVED:

F. L. Cain, Director
Electronics and Computer Systems Laboratory

A-3055

ENGINEERING EXPERIMENT STATION
GEORGIA INSTITUTE OF TECHNOLOGY • ATLANTA, GEORGIA 30332

November 18, 1981

Department of the Air Force
Air Force Systems Command
Aeronautical Systems Division/PMRNB
Wright-Patterson AFB, OH 45433

Attention: Mr. James H. Merritt, USAFSAM/RZP

Reference: Contract No. F33615-81-K-0620
Operational Evaluation of New 435-MHz RFR Facility
Georgia Tech Project A-3055

Subject: Performance and Cost Report No. 2
Reporting Period: 1 October 1981 through 31 October 1981

Gentlemen:

Performance and cost information for the referenced contract are presented below for the reporting period 1 October 1981 through 31 October 1981.

I. Performance Information

As part of the design and construction of the circular, parallel-plate assembly in the Radiation Room in the RFR Facility, shaped nylon blocks with mounting holes were fitted inside the four slotted-cylinder antennas to provide a means for attaching the coaxial feed cables across the slots. During checkout of the facility, feed cables were connected to the four outputs on the MICON transmitter. After operating the transmitter at full power for extended time periods, it was noted that the forward and reflected power levels began to vary in a manner that was unpredictable and that differed from one output to the next. Tests using dummy loads revealed that this was not caused by either the transmitter or that part of the cabling external to the slotted-cylinder antennas. A light was then coupled into the slotted cylinders, and a visual inspection indicated that the nylon mounting blocks were apparently being heated to the point of slowly melting during operation of the facility. Calculations of the dielectric properties of nylon supported this possibility, so plans to disassemble the circular, parallel-plate structure were made and Teflon was identified as a material not likely to be heated while supporting the feed cable. A cylinder of Teflon was ordered, and, during this reporting period, a procedure was devised by which the slotted-cylinder antennas could be worked up through the false ceiling in the Radiation Room without disassembly of the plates. The removal of antennas was accomplished, and the baluns plus mounting blocks were removed. Examination revealed that the primary problem was heating of the dielectric material

in the cable that connects the baluns to the mounting blocks. This heating must result from standing waves between the baluns and mounting blocks. The new Teflon mounting blocks have been installed in the cylinder antennas and techniques for reducing standing waves are being investigated.

Since the system was inactivated by the problem with cable dielectric and mounting blocks, it was decided that the slotted-cylinder antennas in the Control Room should be removed from their plate assembly. This removal permitted Teflon blocks to be mounted in these antennas in anticipation that, at some future date, these plates may be used to radiate experimental animals. Such a capability would be important if bioeffects due to radiation are observed during exposures in the current Radiation Room.

Bid information from 11 different cage washer manufacturers have been obtained and compiled such that information in the following categories can be easily cross-referenced: delivery, warranty, service, life expectancy, cycles, baskets, washing system design, utility requirements, size, installation, and cost. Based on this information, a washer from either Southern Cross or CESCO appears best. The Southern Cross unit washes at 140°F, uses a chemical disinfectant, and requires no ventilation in the room to remove excess humidity. In contrast, the CESCO unit washes at 180°F, can apply a disinfectant if desirable, and would almost certainly require room ventilation for humidity removal. A list of users for each washer is now being obtained from the two manufacturers and they will be contacted during the next reporting period. Prices of $13,237 and $14,787, respectively, for the Southern Cross and CESCO washers include the basic washing machine plus water heater, cage rack, bottle rack, and entry/exit tables.

II. Cost Information

a. Costs incurred during this reporting period:

Personal Services	$2,914.48
Fringe Benefits	289.05
Overhead	1,840.71
Materials and Supplies	143.22
Equipment	0.00
Travel	0.00
Computer Time	0.00
Emory Subcontract	0.00
TOTAL	$ 5,187.46

A-3055

ENGINEERING EXPERIMENT STATION
GEORGIA INSTITUTE OF TECHNOLOGY • ATLANTA, GEORGIA 30332

January 11, 1982

Department of the Air Force
Air Force Systems Command
Aeronautical Systems Division/PMRNB
Wright-Patterson AFB, OH 45433

Attention: Mr. James H. Merritt, USAFSAM/RZP

Reference: Contract No. F33615-81-K-0620
Operational Evaluation of a New 435-MHz RFR Facility
Georgia Tech Project A-3055

Subject: Performance and Cost Report No. 3
Reporting Period: 1 November 1981 to 1 December 1981

Gentlemen:

Performance and cost information for the referenced contract are presented below for the reporting period 1 November 1981 to 1 December 1981.

I. Performance Information

The major technical activity on the project during this reporting period involved finalizing the order for the cage washer and data acquisition system plus further efforts toward installation of the cable mounting blocks in the slotted-cylinder antennas. Additionally, progress on the project was reviewed in detail with Dr. Dave Erwin, SAM/RZP, during his visit to Georgia Tech on 17-18 November 1981.

As noted in the last Performance and Cost Report, the choice of a cage washer was reduced to units from Southern Cross and CESCO. In response to requests to both manufacturers, lists of facilities using Southern Cross and CESCO washers were obtained. Telephone contacts with responsible persons at these facilities resulted in highly complimentary statements regarding operation and maintenance of washers from both manufacturers. Of specific interest was the fact that the chain-driven wash heads which move back and forth on a track in the CESCO washer had apparently provided no maintenance problems.

Since responses from users of washers from the two manufacturers were equally favorable, the purchase decision was based on cost and the extent of modifications required in the Radiofrequency Radiation (RFR) facility to accept the washer. The Southern Cross washer was approximately $1000 less expensive and did not require removal of door facings to move it into the washer room; therefore, it was ordered at a total

cost of $13,349. A memorandum was then prepared requesting that a 0.75-inch copper hot water line, a three-phase 115-volt power line, and a drain be installed in the washer room.

In defining an adequate data acquisition system for the project, two major alternatives were considered. One alternative involved purchase of individual components of the system from a variety of sources, followed by assembly of these components into a functioning system and development of the necessary software. The second alternative involved purchase of a complete data acquisition system with associated software. The first alternative is significantly less expensive in terms of hardware purchases, but would require an appreciable amount of effort to properly assemble the total system and develop the software. In contrast, hardware costs for the second alternative are significant, but operational status could be achieved with a minimal additional effort and considerable software is already available at no or reduced cost. In deciding between the two alternatives, the microcomputer systems used at the University of Washington RFR Facility and at SAM/RZP were carefully considered.

In the final analysis, the second alternative was selected and data from computer manufacturers was obtained. Performance requirements dictated that the data acquisition system (1) provide an automated data logging capability, (2) continuously monitor certain performance parameters of the radiation facility, (3) perform statistical analysis of the data, (4) communicate with the Georgia Tech CYBER 70/74 computer to allow data transfer for further processing, and (5) utilize the S-100 bus system for design flexibility. During the next reporting period, a vendor for the data acquisition system will be identified.

On 17-18 November 1981, Dr. Dave Erwin of SAM/RZP visited Georgia Tech to review the status of SAM projects in the Engineering Experiment Station. For this project, the various radiation concepts considered during the initial analyses were reviewed and the technical advantages of the circular, parallel-plate concept were discussed in detail. The eight-room RFR facility being constructed under SAM sponsorship was toured and earlier problems with overheating of baluns and cables were discussed. The cage design was reviewed and facilities in the Surgery Lab, Anechoic Chamber, and Compact Range were toured. In Dr. Popovic's Laboratory at Emory University, the cannulated rat as a model for future bioeffects studies was discussed, cannulation techniques were demonstrated, and an offer to train SAM/RZP technicans to cannulate rats was extended. No redirections resulted from this project review.

II. Cost Information

a. Costs incurred during this reporting period:

Personal Services	$1,370.95
Fringe Benefits	158.90
Overhead	913.36
Materials and Supplies	130.81
Equipment	0.00
Travel	0.00
Computer Time	0.00
Emory Subcontract	0.00
TOTAL	$ 2,574.02

b. Cumulative costs through this reporting period:

Personal Services	$5,906.49
Fringe Benefits	628.06
Overhead	3,744.71
Materials and Supplies	274.03
Equipment	0.00
Travel	0.00
Computer Time	0.00
Emory Subcontract	27,208.35
TOTAL	$37,761.64

c. Amount vouchered to ACO: $34,969.83

d. Estimated funds necessary to complete project: $78,900.

Respectfully submitted,

J. C. Toler
Project Director

APPROVED:

F. L. Cain, Director
Electronics and Computer Systems Laboratory

A-3055

ENGINEERING EXPERIMENT STATION
GEORGIA INSTITUTE OF TECHNOLOGY • ATLANTA, GEORGIA 30332

January 26, 1982

Department of the Air Force
Air Force Systems Command
Aeronautical Systems Division/PMRNB
Wright-Patterson AFB, OH 45433

Attention: Mr. James H. Merritt, USAFSAM/RZP

Reference: Contract No. F33615-81-K-0620
Operational Evaluation of a New 435-MHz RFR Facility
Georgia Tech Project A-3055

Subject: Performance and Cost Report No. 4
Reporting Period: 1 December 1981 to 1 January 1982

Gentlemen:

Performance and cost information for the referenced contract are presented below for the reporting period 1 December 1981 to 1 January 1982.

I. Performance Information

As discussed in the last Performance and Cost Report, a cage washer has been ordered from Southern Cross Manufacturing Corporation, Chambersburg, PA at a cost of $13,349. During this reporting period, telephone conversations with Southern Cross representatives established the washer delivery date as 4 January 1982. In-house arrangements have been made to have an electrician and plumber available on 4 January 1982 so the washer can be connected to utilities and checked out prior to its acceptance. Provisions for the required drain and three-phase power line in the washer room of the Radiofrequency Radiation (RFR) Facility were completed; however, the required hot water line is currently not installed. If this water line is not installed prior to delivery of the washer, alternate means for providing hot water will have to be used.

During this reporting period, a vendor and specific items of equipment were identified for the data acquisition system. The vendor is Systems Atlanta, Inc. and the equipment items are as follows:

- Cromemco System II Computer
  With two five-inch floppy disk drives providing 780 kbytes of storage and 64 kbytes of memory

- Cromemco D + 7A Input/Output Card
  Seven-channel A/D and D/A

- Cromemco Clock-Calender Card
  Real time clock

- Adds Viewpoint CRT
  Data Display

- Epson MX-80 Printer
  Provides hard copy of data

- Pickles and Trout PT-488 IEEE Bus Interface
  For communicating with the electronic balance

- Cromemco Input/Output Interface
  Adds more I/O ports to the two provided by the computer

- Cromemco Structured Basic

- 19-inch enclosure with cables and support hardware

Currently, a cost estimate is being obtained from Systems Atlanta.

II. Cost Information

a. Costs incurred during this reporting period:

Personal Services	$3,423.89
Fringe Benefits	387.47
Overhead	2,111.85
Materials and Supplies	28.37
Equipment	15,510.93
Travel	0.00
Computer Time	0.00
Emory Subcontract	0.00
TOTAL	$21,462.51

b. Cumulative costs through this reporting period:

Personal Services	$9,330.38
Fringe Benefits	1,015.53
Overhead	5,856.56
Materials and Supplies	302.40
Equipment	0.00
Travel	0.00
Computer Time	0.00
Emory Subcontract	27,208.35
TOTAL	$43,713.22

c. Amount vouchered to ACO: $35,910.87

d. Estimated funds necessary to complete project: $72,300.

Respectfully submitted,

J. C. Toler
Project Director

APPROVED:

F. L. Cain, Director
Electronics and Computer Systems Laboratory

A-3055

ENGINEERING EXPERIMENT STATION
GEORGIA INSTITUTE OF TECHNOLOGY • ATLANTA, GEORGIA 30332

February 26, 1982

Department of the Air Force
Air Force Systems Command
Aeronautical Systems Division/PMRNB
Wright-Patterson AFB, OH 45433

Attention: Mr. James H. Merritt, USAFSAM/RZP

Reference: Contract No. F33615-81-K-0620
Operational Evaluation of a New 435-MHz RFR Facility
Georgia Tech Project A-3055

Subject: Performance and Cost Report No. 5
Reporting Period: 1 January 1982 to 1 February 1982

Gentlemen:

Performance and cost information for the referenced contract are presented below for the reporting period 1 January 1982 to 1 February 1982.

I. Performance Information

As noted in the previous Performance and Cost Report, the cage washer from Southern Cross Manufacturing Corporation was scheduled for delivery on 4 January 1982. This delivery date was not met and, in fact, two other delivery dates established by the manufacturer's representative were not met during the month. Conversations with the manufacturer indicate that bad weather has delayed construction and checkout of the washer, and the date of 2 February 1982 has now been established as the delivery date. These delays are not only causing problems with the project schedule, but it has become increasingly difficult to schedule maintenance people to be available for connecting electricity and water to the washer.

Cost estimates were obtained from Systems Atlanta, Inc. for the several components that make up the data acquisition system. These estimates were as follows:

• Cromemco II System	$4,695.00
• Cromemco D + 7A I/O	295.00
• Cromemco Clock/Calender Card	175.00
• Adds Viewpoint CTR	645.00
• Epson MX-80 Printer	645.00
• Cromemco Tu-Art I/O Interface	345.00
• Pickles and Trout IEEE-488 Interface	450.00
• Equipment Rack and Support Hardware	500.00
• Interconnect Cables	N/C
• Cromemco BASIC	N/C
	$7,750.00

Based on this estimate, a purchase order was initiated for the data acquisition system. Another purchase order was intiated for components to construct the receivers that will be located in the Radiation Room and used to monitor the exposure field.

In Performance and Cost Report No. 2, the problem with melting of the nylon mounting blocks used to support feed cables for the slotted-cylinder antennas was discussed. These blocks have now been replaced with ones made of Teflon; therefore, the effect of the problem (melting) was resolved, but the cause (standing waves) remained unchanged. To remedy this situation, an air-line balun made of brass was designed to accommodate high power levels while matching the transmitter output to the terminal impedance of the slotted cylinder antenna. During this reporting period, the machine shop completed one of these baluns and the antennas were again removed from the circular, parallel plate structures. The balun was mounted inside the cylindrical antenna and efforts to tune the assembly to 435 MHz were begun. These efforts are using a network analyzer, and after the tuning is completed, the assembly will be repositioned in the circular, parallel plates. High power levels will then be coupled to the assembly while heat build-up and standing waves are monitored.

II. Cost Information

a. Costs incurred during this reporting period:

Personal Services	$2,117.53
Fringe Benefits	245.41
Overhead	1,300.71
Materials and Supplies	1.98
Equipment	1,211.93
Travel	0.00
Computer Time	0.00
Emory Subcontract	0.00
TOTAL	$ 4,877.56

b. Cumulative costs through this reporting period:

Personal Services	$11,447.91
Fringe Benefits	1,260.94
Overhead	7,157.27
Materials and Supplies	5,304.38
Equipment	16,722.86
Travel	0.00
Computer Time	0.00
Emory Subcontract	27,208.35
TOTAL	$69,101.71

c. Amount vouchered to ACO: $67,889.78

d. Estimated funds necessary to complete project: $48,772.

Respectfully submitted,

J. C. Toler
Project Director

APPROVED:

F. L. Cain, Director
Electronics and Computer Systems Laboratory

A-3055

ENGINEERING EXPERIMENT STATION
GEORGIA INSTITUTE OF TECHNOLOGY • ATLANTA, GEORGIA 30332

March 11, 1982

Department of the Air Force
Air Force Systems Command
Aeronautical Systems Division/PMRNB
Wright-Patterson AFB, OH 45433

Attention: Mr. James H. Merritt, USAFSAM/RZP

Reference: Contract No. F33615-81-K-0620
Operational Evaluation of a New 435-MHz RFR Facility
Georgia Tech Project A-3055

Subject: Performance and Cost Report No. 6
Reporting Period: 1 February 1982 to 1 March 1982

Gentlemen:

Performance and cost information for the referenced contract are presented below for the reporting period 1 February 1982 to 1 March 1982.

I. Performance Information

As noted in Performance and Cost Report No. 5, an air-line balun made of brass was designed to both accommodate the output power level of the transmitter and match the transmitter output impedance to the terminal impedance of the slotted-cylinder antenna. This design is shown in the attached figure. During this reporting period, efforts to tune the balun to 435 MHz were completed and three more baluns of the same design were ordered from the Main Machine Shop. The tuning was accomplished by varying the length of the two slots shown in the figure. These slots are approximately 0.125 inches wide and spaced 180 degrees apart. To vary (shorten) the slot length, conductive filler material is taped into position at the proximal end of the slots, and, since slot length influences the frequency of operation, tuning is accomplished. Using a network analyzer and with the balun mounted in the slotted-cylinder antenna, a -8 dB match was obtained at 435 MHz. This match can be improved upon, but it was sufficient to justify ordering additional baluns of the same design. Receipt of these baluns from the Main Machine Shop is expected during the next reporting period. (It is noted that one of the three baluns ordered will be somewhat larger because it must accommodate the combined output power from all four amplifiers in the transmitter.) The center conductor in the balun is maintained in position by three Teflon supports located as shown in the attached figure. This center conductor plus the second conductor fixed to the outside wall of the balun form the pair of conductors that fit into the Teflon mounting blocks and feed the antenna.

The cage washer from Southern Cross Manufacturing Corporation was received, connected to building utilities, and checked out during this reporting period; however, the rack in which four complete cages are to be placed during the wash cycle will not accept the cages and their tops. Therefore, it is to be rebuilt and shipped later. The water temperature can be varied over a range of 130° to 180°F, and liquid disinfectant can be added to the wash cycle. The automatic timers and reset cycles have the following durations:

Wash Timer: 0 - 10 minutes
Tap Rinse Timer: 0 - 10 minutes
Final Rinse Timer: 0 - 1 minute

After installation and acceptance of the washer, Southern Cross was asked to provide a recommended list of spare parts that should be maintained on hand to prevent a long down-time in the event of a washer failure during an experiment. The following parts were identified:

2 Cog Belts	$ 71.02
2 SPDT Relays	42.24
2 Shaft Cog Pulleys	138.56
2 Hub Cog Pulleys	194.40
1 Nylon Pillow Block	48.96
1 10-Minute Timer	189.32
1 1-Minute Timer	175.30
1 3/4" Treaded Solenoid Value	186.00
	$1,045.80

With these parts available, a rapid repair should be possible for almost any conceivable failure mode.

II. Cost Information

a. Costs incurred during this reporting period:

Personal Services	$2,645.16
Fringe Benefits	306.56
Overhead	2,528.94
Materials and Supplies	959.42
Equipment	0.00
Travel	0.00
Computer Time	0.00
Emory Subcontract	0.00
TOTAL	$ 6,440.08

b. Cumulative costs through this reporting period:

Personal Services	$14,093.07
Fringe Benefits	1,567.50
Overhead	9,686.21
Materials and Supplies	20,774.73
Equipment	0.00
Travel	0.00
Computer Time	0.00
Emory Subcontract	27,208.35
TOTAL	$73,329.86

c. Amount vouchered to ACO: $72,804.86

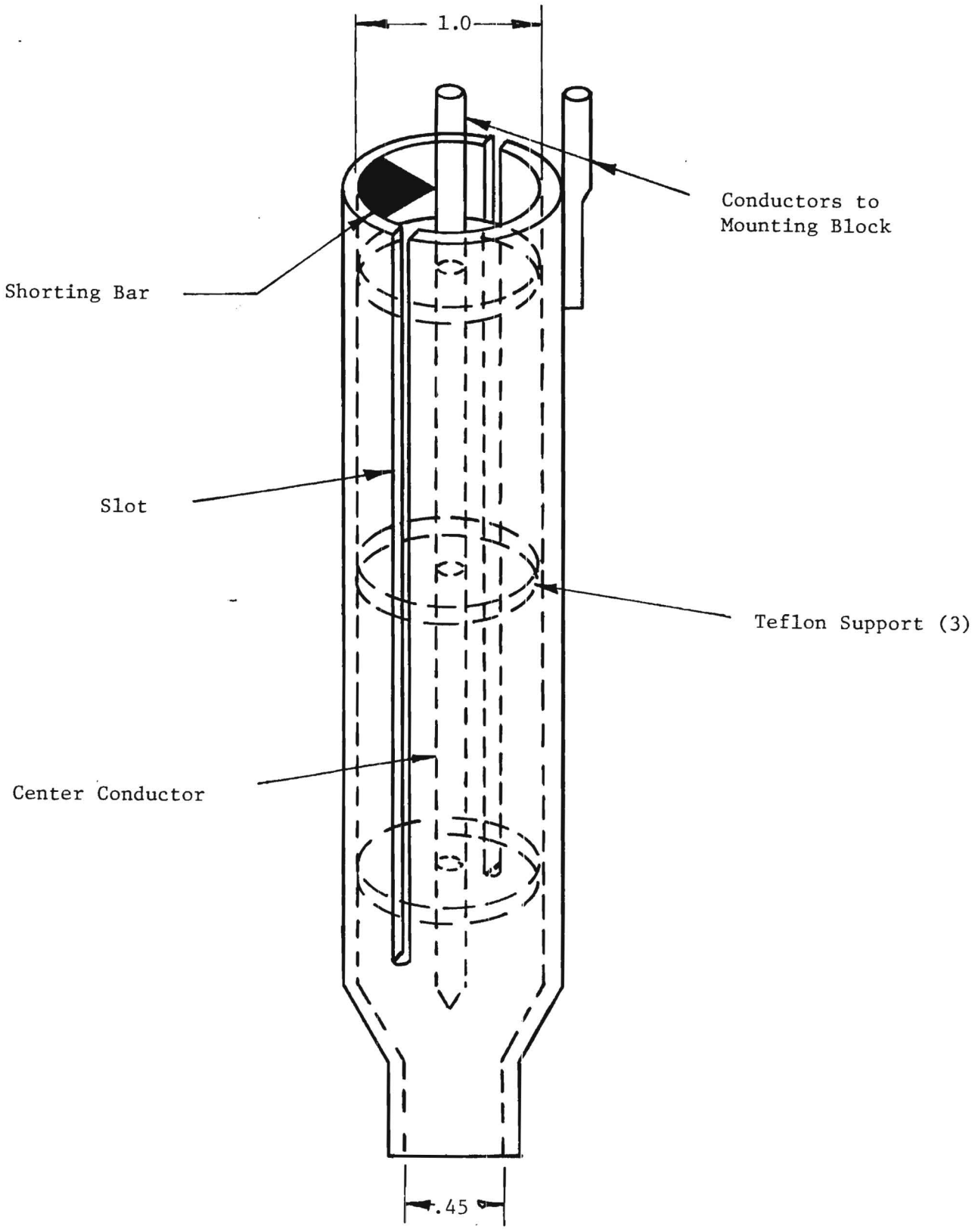
d. Estimated funds necessary to complete project: $43,397.

Respectfully submitted,

J. C. Toler
Project Director

APPROVED:

F. L. Cain, Director
Electronics and Computer Systems Laboratory


1.0
Conductors to
Mounting Block
Shorting Bar
Slot
Teflon Support (3)
Center Conductor
.45

A-3055

Georgia Institute of Technology
ENGINEERING EXPERIMENT STATION
ATLANTA, GEORGIA 30332

April 21, 1982

Department of the Air Force
Air Force Systems Command
Aeronautical Systems Division/PMRNB
Wright-Patterson AFB, OH 45433

Attention: Mr. James M. Merritt, USAFSAM/RZP

Reference: Contract No. F33615-81-K-0620
Operational Evaluation of a New 435-MHz RFR Facility
Georgia Tech Project A-3055

Subject: Performance and Cost Report No. 7
Report Period: 1 March 1982 to 1 April 1982

Gentlemen:

Performance and cost information for the referenced contract are presented below for the reporting period 1 March 1982 to 1 April 1982.

I. Performance Information

As noted in Performance and Cost Report No. 6, three additional airline baluns were ordered from the Main Machine Shop for use in matching the transmitter output impedance to the terminal impedance of the slotted-cylinder antenna. These baluns have now been received and connected to the Teflon mounting blocks inside the four antennas. Tuning of the balun-antenna assembly is underway using a network analyzer.

The rack in which Plexiglas cages and cage tops are to be positioned in the cage washer was received during this reporting period. With receipt of this rack, the cage washer is complete and its installation/operation has been accepted.

The 200 cages for housing rats during long-term low-level experiments will be positioned on eight Styrofoam rings that fit on the periphery of the eight circular, parallel-plate antennas. The rings must have an outer diameter of 12 feet, a height of 4 inches, and a width of 7.75 inches. The Styrofoam material for these rings was ordered during this reporting period and a template to be used in cutting the rings was constructed. A commercial organization will use the template to cut the eight rings.

It is anticipated that the initial experiments using the RFR Facility will include several hormones among the biological endpoints that will be monitored for indications of bioeffects. Access to these endpoints will be by means of blood drawn via an implanted cannula in the rats, and blood samples will need to be drawn frequently to assure that bioeffects are not

missed; therefore, it is necessary that microquantity blood samples be drawn to preclude the introduction of artifacts resulting from removal of excessive amounts of the total blood volume. During this reporting period, further efforts have been expended in developing adequate assays for corticosterone, ACTH, and prolactin using the microquantity blood samples. These assays are based on radiometric techniques (radioimmunoassay) that involve competition between unknown levels of the unlabeled hormone being measured and a fixed amount of labeled hormone for a limited number of binding sites of specific antibodies. Known quantities of unlabeled hormones are varied in order to obtain a standard curve and unknown amounts of the analyzed hormone are determined by interpolation with the generated standard curve. The procedure specifically measures quantities of protein or a small hapten in the nanogram or picogram range and allows the simultaneous determination of many samples. Antibodies are usually produced in rabbits, goats or guinea pigs by repeated injections of antigen (immunogen). These raised antibodies are then used in the immunoassay to determine small amounts of substances in biological fluids. The principle has been used successfully for a number of years for the measurement and study of polypeptides, hormones and steroids. Because of their simplicity, these assay techniques are applicable to the analysis of a large number of samples. They are also rapid, sensitive, specific, reliable and require a minimum quantity of blood or urine, rendering them suitable for use with small samples. This feature is very important when repetitive sampling is required in a particular study.

Currently, three groups of six rats each are being used to develop the assay techniques. Assays are being conducted at 20 minute and weekly intervals to define data accuracy and reproducibility. The individual assays use microsample blood volumes from the rats, and all rats have indwelling cannulae. Results from these efforts will be available during the next reporting period.

II. Cost Information

a. Cost incurred during this reporting period:

Personal Services	$1,982.91
Fringe Benefits	229.80
Overhead	11,869.84
Material and Supplies	19,368.82
Equipment	0.00
Travel	0.00
Computer Time	0.00
Emory Subcontract	0.00
TOTAL	$33,451.37

b. Cumulative costs through this reporting period:

Personal Services	$16,075.98
Fringe Benefits	1,797.30
Overhead	21,556.05
Materials and Supplies	21,319.55
Equipment	0.00
Travel	0.00
Computer Time	0.00
Emory Subcontract	27,208.35
TOTAL	$87,957.23

c. Amount vouchered to ACO: $87,957.23

d. Estimated funds necessary to complete project: $28,245.

Respectfully submitted,

J. C. Toler
Project Director

APPROVED:

F. L. Cain, Director
Electronics and Computer Systems Laboratory

A-3055

Georgia Institute of Technology
ENGINEERING EXPERIMENT STATION
ATLANTA, GEORGIA 30332

June 10, 1982

Department of the Air Force
Air Force Systems Command
Aeronautical Systems Division/PMRNB
Wright-Patterson AFB, OH 45433

Attention: Mr. James M. Merritt, USAFSAM/RZP

Reference: Contract No. F33615-81-K-0620
Operational Evaluation of a New 435-MHz RFR Facility
Georgia Tech Project A-3055

Subject: Performance and Cost Report No. 8
Report Period: 1 April 1982 to 1 May 1982

Gentlemen:

Performance and cost information for the referenced contract are presented below for the reporting period 1 April 1982 to 1 May 1982.

I. Performance Information

Essentially all technical efforts during this reporting period were concerned with preparing for and initiating the six-week Shakedown Evaluation of the RFR Facility. Typical efforts were as follows:

- Tuning of the balun/antenna assemblies for the four slotted-cylinder antennas was completed using the network analyzer, and the assemblies were installed in the four-tier stack of circular, parallel plates. During the installation, the center and outer conductors of two of the baluns pulled loose from their Teflon mounting blocks inside the slotted cylinders, and had to be removed, reconnected, retuned, and installed in the circular, parallel plates again. This occurred because of the considerable strain on the balun/Teflon block connection point induced by movement during installation of the rather long semi-rigid coaxial cables connected to the baluns. In view of this situation, there is concern with the design adequacy of the mounting configuration, and plans are being made to investigate this area after completion of the Shakedown Evaluation.

- Using the small E-field probes on loan from the Bureau of Radiological Health, the uniformity of the exposure field was measured at 45 degrees increments around the circular, parallel plates. As was the case for the prototype plates, the azimuth plane E-field varied 0.75 dB or less for each set of plates.

- The Cromemco Data Acquisition System was received and checked out, then programed to accept data on each of the 100 rats in the Shakedown Evaluation. However, it was learned that the module purchased with the Satorius electronic balance for the purpose

of interfacing with the computer was the wrong model. It has been returned to Satorius and the correct model has been ordered. In the meantime, weight data for each rat is being manually entered into the computer. In its final configuration, the average of ten weights measured over a 300 millisecond period will automatically be entered into the computer along with a keyboard-entered identification code of each rat.

- The 150 Spraque-Dawley rats required for this study completed their quarantine at Emory University and 127 were cannulated during this reporting period. After recovery from cannulation, 100 rats were selected for inclusion in the Shakedown Evaluation and moved to Georgia Tech where a one-week period was provided for acclimation to the new surroundings and to the Plexiglas cages.

- Final shelving, desks, tables, workbenches, sink, etc. were obtained and set in place.

After completion of tasks such as those above, the Shakedown Evaluation was begun on 28 April 1982; however, blood samples were not drawn the first week, so the Evaluation will extend seven weeks to permit blood to be drawn six times. Two of the four antennas were energized and provided an exposure field of 1.0 mW/cm^2 for the 50 radiated rats. During the first few days of the Evaluation, several problems arose and were resolved. These included:

- The Plexiglas cages had a tendency to tilt forward when the rats stood on their hind legs and leaned toward the cage front. This resulted from a recent design change made to facilitate servicing of the cages. The design change moved the water bottle and food hopper from the cage ends to the cage front. To solve the problem, the 8-ounce water bottles were exchanged for 4-ounce bottles (a rat drinks approximately 1.2 ounces per day) and the food hoppers are not completely filled.

- The Plexiglas cage tops and bases vary only a small amount in size, but this variation was enough that some tops could be worked off the cages by the rats. On four occasions, a rat knocked a cage over while climbing out of it. This problem was resolved by custom selecting tops that fit the cages.

- The Styrofoam trays used under each cage to collect wastes are shallow enough that feces are knocked out of the tray and onto the floor by movement of the rat. Efforts are being made to obtain waste collection trays that have a higher rim.

Efforts continued at Emory University to finalize the assay for ACTH, prolactin, corticosterone, catecholamines, and growth hormone. In the case of ACTH, prolactin, and corticosterone, the range of normal variability has been defined for the assays (see Tables I, II, and III) and the reference point above which an effect is indicated is now being established.

II. Cost Information

a. Cost incurred during this reporting period:

Personal Services	$ 199.75
Fringe Benefits	23.14
Overhead	850.95
Material and Supplies	8,479.24
Equipment	0.00
Travel	0.00
Computer Time	0.00
Emory Subcontract	0.00
TOTAL	$ 9,553.08

b. Cumulative costs through this reporting period:

Personal Services	$16,275.73
Fringe Benefits	1,820.44
Overhead	22,407.00
Materials and Supplies	30,298.84
Equipment	0.00
Travel	0.00
Computer Time	0.00
Emory Subcontract	27,208.35
TOTAL	$98,010.36

c. Amount vouchered to ACO: $90,855.36

d. Estimated funds necessary to complete project: $18,716.

Respectfully submitted,

J. C. Toler
Project Director

APPROVED:

F. L. Cain, Director
Electronics and Computer Systems Laboratory

TABLE I

PLASMA CONCENTRATIONS OF CORTICOSTERONE AT 0 MINUTE (A) AND 20 MINUTE (B) TIME INTERVALS FROM THE SAME RESTING RATS WITH CHRONICALLY IMPLANTED AORTIC CANNULAE

RAT	CORTICOSTERONE (µg/dl)
821A	68
821B	63
822A	71
822B	65
823A	53
823B	42
824A	--
824B	33
827A	18
827B	44
828A	49
828B	58

TABLE II

PLASMA CONCENTRATIONS OF ACTH AT 0 MINUTE (A) AND 20 MINTUE (B) TIME INTERVAL FROM THE SAME RESTING RATS WITH CHRONICALLY IMPLANTED AORTIC CANNULAE

RAT	ACTH (pg/ml)
821A	256
821B	232
822A	276
822B	244
823A	216
823B	216
824A	208
824B	264
827A	250
827B	160
828A	368
828B	280

TABLE III

PLASMA CONCENTRATIONS OF PROLACTIN AT 0 MINUTE (A) AND 20 MINUTE (B) TIME INTERVALS FROM THE SAME RESTING RATS WITH CHRONICALLY IMPLANTED AORTIC CANNULAE

RAT	PROLACTIN (ng/ml)
821A	51
821B	51
822A	83
822B	24
823A	6
823B	1
824A	58
824B	26
827A	4
827B	14
828A	92
828B	27

A-3055

ENGINEERING EXPERIMENT STATION
Georgia Institute of Technology
A Unit of the University System of Georgia
Atlanta, Georgia 30332

June 25, 1982

Department of the Air Force
Air Force Systems Command
Aeronautical Systems Division/PMRNB
Wright-Patterson AFB, OH 45433

Attention: Mr. James M. Merritt, USAFSAM/RZP

Reference: Contract No. F33615-81-K-0620
Operational Evaluation of a New 435-MHz RFR Facility
Georgia Tech Project A-3055

Subject: Performance and Cost Report No. 9
Report Period: 1 May 1982 to 1 June 1982

Gentlemen:

Performance and cost information for the referenced contract are presented below for the reporting period 1 May 1982 to 1 June 1982.

I. Performance Information

All technical efforts during this reporting period were concerned with performance of the Shakedown Evaluation on the Radiofrequency Radiation (RFR) Facility. This Evaluation began on 28 April 1982 and continued throughout this reporting period. The performance procedure that evolved during the month was as follows:

- On Mondays of each week, Styrofoam soil trays under the cages were replaced, all water bottleds were refilled, rat chow was added to the food hoppers as necessary, blood was drawn from the 50 cannulated rats (25 exposed, 25 sham exposed) and all rats weighed.

- On Wednesdays of each week, the Styrofoam soil trays under the cages were replaced, all water bottles were refilled, rat chow was added to the food hoppers as necessary, and all Plexiglas cages and water bottles were washed.

- On Fridays of each week, Styrofoam soil trays under the cages were replaced, all water bottles were refilled, and rat chow was added to the food hoppers as necessary.

- On each working day of the week, the Control and Radiation Rooms were swept and, on Tuesdays and Thursdays, floors in these rooms were mopped with a combination disinfectant and deodorizer.

When not involved in the above activities, project personnel were engaged in other tasks that included (1) entering weight data in the Data Acquisition System, (2) developing programs for the Data Acquisition System, (3) repairing broken Plexiglas cages, (4) monitoring performance of the transmitter, (5) plotting weight curves for each rat, etc. Some observations made during the month were as follows:

- Cages housing the rats during the Shakedown Evaluation are made of Plexiglas sides with tops and bottoms constructed of closely-spaced glass rods mounted in Plexiglas frames. The result is a cage transparent to RFR and excellent for viewing the status of individual rats; however, it is also a rather fragile cage that breaks apart when dropped. Further, the small Plexiglas strips on the cage sides used as guides over which the tops are fitted tend to break off easily. As a result, several cages were disabled each day during which cages were washed. The most frequent problems involved breaking off one or both of the Plexiglas guides used to fit tops onto the cages and breaking cage tops by dropping them. Other than being much more careful, no solution to this problem exists at this time. It is noted that glass rods in the cage tops and bottoms are necessary because of the tendency of rats to gnaw through plastic rods. Also, a design change for the Plexiglas strips used to guide tops onto the cages will be difficult because 250 cages using this design now exist.

- Odor control continues to be a concern in a multistory building where installation of another animal housing facility was not welcomed because of the possibility that unpleasant smells could get into heating and air conditioning system. Stringent attention has been given to cleanliness and frequent use has been made of a disinfectant/deodorizer. During subsequent experiments, a deodorizing paper mat (Shepherd Speciality Papers, Inc., Kalamazoo Mich.) will be used in the soil trays under each cage.

- With extensive efforts on Fridays, the rats can be satisfactorily maintained over the weekend without project personnel having to be on-site. The extensive efforts involve assuring that water bottles and food hoppers are completely filled and that the facility is throughly cleaned. However, with a full complement of 200 rats, it may prove necessary to have project personnel work a six-day week to assure that all animals are properly cared for.

- Entering weight data into the Data Acquisition System and plotting weight curves for all rats is a time-consuming task. Upon receipt of the module for interfacing the electronic balance to the Data Acquisition System, the magnitude of this task will be reduced; however, it will still be desirable to program the Data Acquisition System to automatically plot these data.

On 20 May 1982, Mr. John Mitchell and Mr. Jim Merritt of the Air Force School of Aerospace Medicine, Brooks AFB, TX, reviewed the project status during meetings at Georgia Tech and Emory University. The RFR Facility and the status of efforts on the Shakedown Evaluation were described. The Facility was then toured and an explanation of procedures used in the Evaluation was provided. During discussions, various ideas relative to follow-on experiments were presented. At Emory University, Dr. V. Popovic's laboratory was toured and a demonstration was given to show how blood is drawn from cannulated rats. No project redirections resulted from the status review.

II. Cost Information

a. Cost incurred during this reporting period:

Personal Services	$1,693.22
Fringe Benefits	196.23
Overhead	5,363.79
Material and Supplies	7,862.89
Equipment	0.00
Travel	0.00
Computer Time	0.00
Emory Subcontract	0.00
TOTAL	$15,116.13

b. Cumulative costs through this reporting period:

Personal Services	$17,968.85
Fringe Benefits	2,016.67
Overhead	27,770.79
Materials and Supplies	30,506.73
Equipment	0.00
Travel	0.00
Computer Time	0.00
Emory Subcontract	27,208.35
TOTAL	$105,471.39

c. Amount vouchered to ACO: $97,816.39.

d. Estimated funds necessary to complete project: $15,500.

Respectfully submitted,

J. C. Toler
Project Director

APPROVED:

F. L. Cain, Director
Electronics and Computer Systems Laboratory

A-3055

ENGINEERING EXPERIMENT STATION
GEORGIA INSTITUTE OF TECHNOLOGY • ATLANTA, GEORGIA 30332

July 23, 1982

Department of the Air Force
Air Force Systems Command
Aeronautical Systems Division/PMRNB
Wright-Patterson AFB, OH 45433

Attention: Mr. James M. Merritt, USAFSAM/RZP

Reference: Contract No. F33615-81-K-0620
Operational Evaluation of a New 435-MHz RFR Facility
Georgia Tech Project A-3055

Subject: Performance and Cost Report No. 10
Report Period: 1 June 1982 to 1 July 1982

Gentlemen:

Performance and cost information for the referenced contract are presented below for the reporting period 1 June 1982 to 1 July 1982.

I. Performance Information

Technical efforts during this reporting period continued to be concerned with the Shakedown Evaluation of the Radiofrequency Radiation (RFR) Facility. This Evaluation concluded on 23 June 1982, eight weeks after it began. Eight weeks were required instead of the contractually-obligated six weeks because blood samples were not drawn on the first and sixth weeks of the Evaluation. During the first week, no blood samples were drawn in order to allow more time for acclimation to the facility environment. The Emory University technician responsible for drawing blood samples was on vacation during the sixth week, so blood samples were not drawn during that period. The Evaluation involved 136 rats (100 rats were contractually required), of which 50 were cannulated. Six rats died during the Evaluation and another one was sacrified because the cannula apparently dislodged from its position in the aortic arch. Micro-samples of blood were drawn from the cannulated rats six times during the Evaluation, each time between 10:00 am and 2:00 pm on Mondays. The blood samples were taken to Emory University, and are being used in the development of reliable and repeatable assays for hormones (catecholamines, growth hormone, ACTH, corticosterone, and prolactin). During the next reporting period, results from these assays will be available and can be incorporated into the final report.

In the last monthly report, it was noted that difficulties were being encountered with the Plexiglas cages used to house rats in the Radiation and Control Rooms. These difficulties involved breaking of the cages during routine handling and washing. During this reporting period, handling procedures have been developed that eliminate most of these difficulties and the damaged cages have been repaired.

II. Cost Information

a. Cost incurred during this reporting period:

Personal Services	$1,606.62
Fringe Benefits	110.21
Overhead	$1,007.28
Materials and Supplies	114.59
Equipment	0.00
Travel	0.00
Computer Time	0.00
Emory Subcontract	11,221.00
TOTAL	$14,059.70

b. Cumulative costs through this reporting period:

Personal Services	$19,575.57
Fringe Benefits	2,126.88
Overhead	28,778.07
Materials and Supplies	30,621.32
Equipment	0.00
Travel	0.00
Computer Time	0.00
Emory Subcontract	34,206.00
TOTAL	$115,307.84

c. Amount vouchered to ACO: $104,086.84

d. Estimated funds necessary to complete project: $1,500.

Respectfully submitted,

J. C. Toler
Project Director

APPROVED:

F. L. Cain, Director
Electronics and Computer Systems Laboratory

A-3055

ENGINEERING EXPERIMENT STATION
GEORGIA INSTITUTE OF TECHNOLOGY • ATLANTA, GEORGIA 30332

October 6, 1982

Department of the Air Force
Air Force Systems Command
Aeronautical Systems Division/PMRNB
Wright-Patterson AFB, OH 45433

Attention: Mr. James M. Merritt, USAFSAM/RZP

Reference: Contract No. F33615-81-K-0620
"Operational Evaluation of a New 435-MHz RFR Facility"
Georgia Tech Project A-3055

Subject: Performance and Cost Report No. 11
Report Period: 1 July 1982 to 1 August 1982

Gentlemen:

Performance and cost information for the referenced contract are presented below for the reporting period 1 July 1982 to 1 August 1982.

I. Performance Information

Technical activities during this reporting period were concerned with preparation of the draft Final Report. This report, which is being authored jointly by Mr. J. Toler of Georgia Tech and Dr. V. Popovic of Emory, first presents summary descriptions of (1) the Radiofrequency Radiation (RFR) Facility used during the Shakedown Evaluation, (2) the data acquisition system, (3) the cage washer, and (4) the electronic balance. The experimental animals used during the Shakedown Evaluation and the protocol used in cannulating these animals are then presented. This is followed by a description of the events that occurred during the Shakedown Evaluation. Finally, a critique is presented which summarizes important conclusions that should be incorporated in subsequent efforts to expose large rodent populations to RFR. This report will be completed during the next performance period.

II. Cost Information

a. Costs incurred during this reporting period:

Personal Services	$ 120.54
Fringe Benefits	23.51
Overhead	88.42
Materials and Supplies	43.27
Equipment	0.00
Travel	0.00
Computer Time	0.00
Emory Subcontract	20,719.09
TOTAL	$20,994.83

A-3055

FINAL TECHNICAL REPORT

GT/EES PROJECT A-3055

OPERATIONAL EVALUATION OF A NEW 435 MHZ RADIOFREQUENCY RADIATION FACILITY

By

J. Toler and V. Popovic

Submitted to

AIR FORCE SCHOOL OF AEROSPACE MEDICINE
Code SAM/RZP
Brooks Air Force Base, TX 78235

Under

Contract No. F33615-81-K-0620

August 1982

GEORGIA INSTITUTE OF TECHNOLOGY

PRELIMINARY

TABLE OF CONTENTS

LIST OF FIGURES

SECTION I

INTRODUCTION

Over the last decade there has been a significant amount of research concerned with whether biological effects, either hazardous or otherwise, are induced in living systems as a result of exposure to radiofrequency radiation. A large portion of this research has involved either engineers or biologists exposing small animal populations to specific pulsed- or continuous-wave radiation environments for relatively short time periods while monitoring biological endpoints presumed to be sensitive to the radiation insult. Although these research efforts have been helpful, there is a very real sense in which they have raised more questions than they have answered. The reasons for this situation are numerous and include the following:

- In many instances, the persons conducting the research have been qualified engineers or biologists, but not both; consequently, key aspects of one discipline or the other were not adequately considered.
- The animal populations have often been too small to yield statistically significant data.
- Exposure facilities have been limited to the point that it has been difficult to study the same biological endpoints during exposure to radiation with the same propagation mode but different electromagnetic parameters.
- No rationale has been provided to explain why the selected endpoints should be responsive to radiofrequency radiation. Also, the procedures used in accessing these endpoints have been capable of masking any radiation-induced bioeffects.
- Dosimetry determinations have not been adequate to accurately characterize the absorbed dose of radiation.
- The exposure frequency and animal species were such that a meaningful extrapolation of the data to the man-model was not possible.

To overcome difficulties such as these, the Air Force School of Aerospace Medicine (AFSAM), Radiation Sciences Division, is sponsoring bioeffects research programs involving interdisciplinary teams exposing large animal populations to real-world radiation environments in facilities versatile enough to generate both pulsed- and continuous-wave fields. The exposure

frequency and animal species are selected to assure extrapolation of the resulting data to the man-model. The endpoints monitored are stress-sensitive and therefore expected to be responsive to effects induced by environmental insults such as radiofrequency radiation.

The AFSAM-sponsored program in the Biomedical Research Division at Georgia Tech's Engineering Experiment Station has involved four individual studies. In the first of these studies [1], several potentially satisfactory facility concepts were analyzed theoretically, and the circular, parallel-plate concept in a multi-tier configuration was identified as the most technically and economically feasible. The second program [2] involved construction of a prototype, single-tier set of circular, parallel plates and development of a slotted cylinder antenna to feed the plates. The plates and antenna were then evaluated by measuring radiation patterns in an anechoic chamber as a function of plate separation, frequency, radial position from the plate center, vertical position between the plates, slot parameters, etc. The third program [3] provided a full-scale, 435-MHz circular, parallel plate radiofrequency radiation facility. The facility consists of two, four-tier stacks of circular, parallel plates located in adjoining, absorber-lined rooms. Additionally, an assay room, transmitter room, utility room, storage room, computer room, and office area, all colocated with the rooms housing the circular, parallel plates, is provided. Each tier of circular, parallel plates accommodates 25 rodents; therefore, one four-tier configuration can be used to expose 100 rodents to a 435-MHz environment while the other configuration can house 100 control rodents. This program also provided Plexiglas cages for the rodents and a 435-MHz transmitter with pulse- and continuous-wave outputs of 5 KW and 200 W, respectively. The fourth program is described in this report and had, as its primary objective, the performance of a "shakedown" evaluation to familarize project personnel with operation of the exposure facility and analysis of the blood samples. This program was undertaken by an interdisciplinary team of engineers from Georgia Tech and medical professionals from Emory University. In addition to performing the shakedown evaluation, this team also integrated several remaining subsystems into the facility and trained biomedical technologists in both cannulation techniques and microassay procedures. Detailed efforts undertaken during this program are described in the following sections of this report.

SECTION II

SUMMARY DESCRIPTION OF FACILITY

Prior to detailed descriptions of efforts related to the shakedown evaluation, it was considered beneficial to provide a summary description of the Radiofrequency Radiation (RFR) Facility. This description will be particularly helpful later in the report when procedures for handling the experimental animals are described. As a key part of the Facility, characteristics of the exposure field in the Radiation Room are also described.

A. Facility Description

The RFR Facility consists of eight rooms located in the basement of the Baker Building on the Georgia Tech main campus. The floor plan and layout for these rooms was specifically designed to provide an essentially self-contained facility for bioeffects studies involving large rodent populations and long-term RFR exposures. The Baker Building is a three-story, brick building in which a large variety of electronic research programs are conducted; however, this program is the only one involving experimental animals. Heating and air conditioning systems for the RFR Facility are isolated from those in the remainder of the Baker Building, and exhaust air is routed outside the building through a large plenum. Power for lights in the rooms that house exposed and sham-exposed animals is routed through a timer that can be programmed to provide any desired lighting cycle. Hot and cold water supplies are taken from primary sources and are therefore unaffected by service outages that might cause loss of water in other Baker Building locations. Rooms housing exposed and sham-exposed animals are constructed in a manner that provides electromagnetic shielding to assure that stray radiation does not reach other research areas. To accomplish this, walls in the Control and Radiation Rooms utilize aluminum-backed sheet rock in their construction. This aluminum backing is joined together with conductive tape along all seams between adjacent panels of sheet rock; therefore, an electrically-conductive aluminum shield is provided for the walls. For the ceilings, individual 2-foot by 2-foot acoustical tiles have been removed, covered with aluminum shielding, and remounted in their metal support frames. Since the facility floor is on ground level, it requires no shielding

The RFR Facility floor plan is shown in Figure 1. During the shakedown evaluation, the rooms designated A and B in Figure 1 housed exposed and sham-exposed rats, respectively. Identifical 12-foot diameter circular, parallel-plate structures were located in both rooms. These structures consisted of four-tier stacks of circular, aluminum plates with an 18-inch separation distance maintained betwen them by 1.5-inch diameter plastic rods [2]. These plates functioned as open-ended waveguides and were fed with slotted cylinder antennas located at the plate centers [3]. The walls of both rooms were also lined identically with pyramidal-shaped microwave absorbing material that provided a -30 dB reflectivity at 500 MHz. The timers were used to cycle the lighting on a 6:00 am - 6:00 pm schedule. Temperature in these rooms was maintained at 70 $\pm$ 2°C during the first four weeks of the evaluation, and then increased to 74 $\pm$ 2°C during the remaining weeks. Humidity was maintained at 48 $\pm$ 2 percent throughout the evaluation. Room C in Figure 1 was used as a storage and maintenance area. Items stored included rat chow, Styrofoam soil trays, custodial supplies, extra cages, etc. The work area in this room was used primarily for repairing damaged cages. The cage washer with its entry and exit tables was housed in Room D. Room E was the area to which the rats were transferred from their large Plexiglas exposure cages into small Plexiglas holding cages in preparation for drawing microsamples of blood. This room also housed the electronic balance and was therefore used when the rats were weighed. After transfer into the small Plexiglas cages, the rats were moved into Room F where a 15-minute or more acclimation period was provided before microsamples of blood were drawn. During this acclimation period, the door to Room F was closed and only the biological technician remained in the room. Room G provided a buffer area between the Control/Radiation Rooms and routine activity in the hallway outside the Facility. It also housed the Data Acquisition System into which weight data for each rat was entered, and desk space for the engineering technician. The 435-MHz transmitter plus spare parts and a work bench were located in Room H. Coaxial connectors on the transmitter top provided power output ports for the four antennas located in the Radiation Room (Room A). Cables connected to these four connectors were routed up through the ceiling of Room H and across to the slotted cylinder antennas in Room A.

The above summary description shows the RFR Facility to be a complex of eight contiguous rooms that provide space for

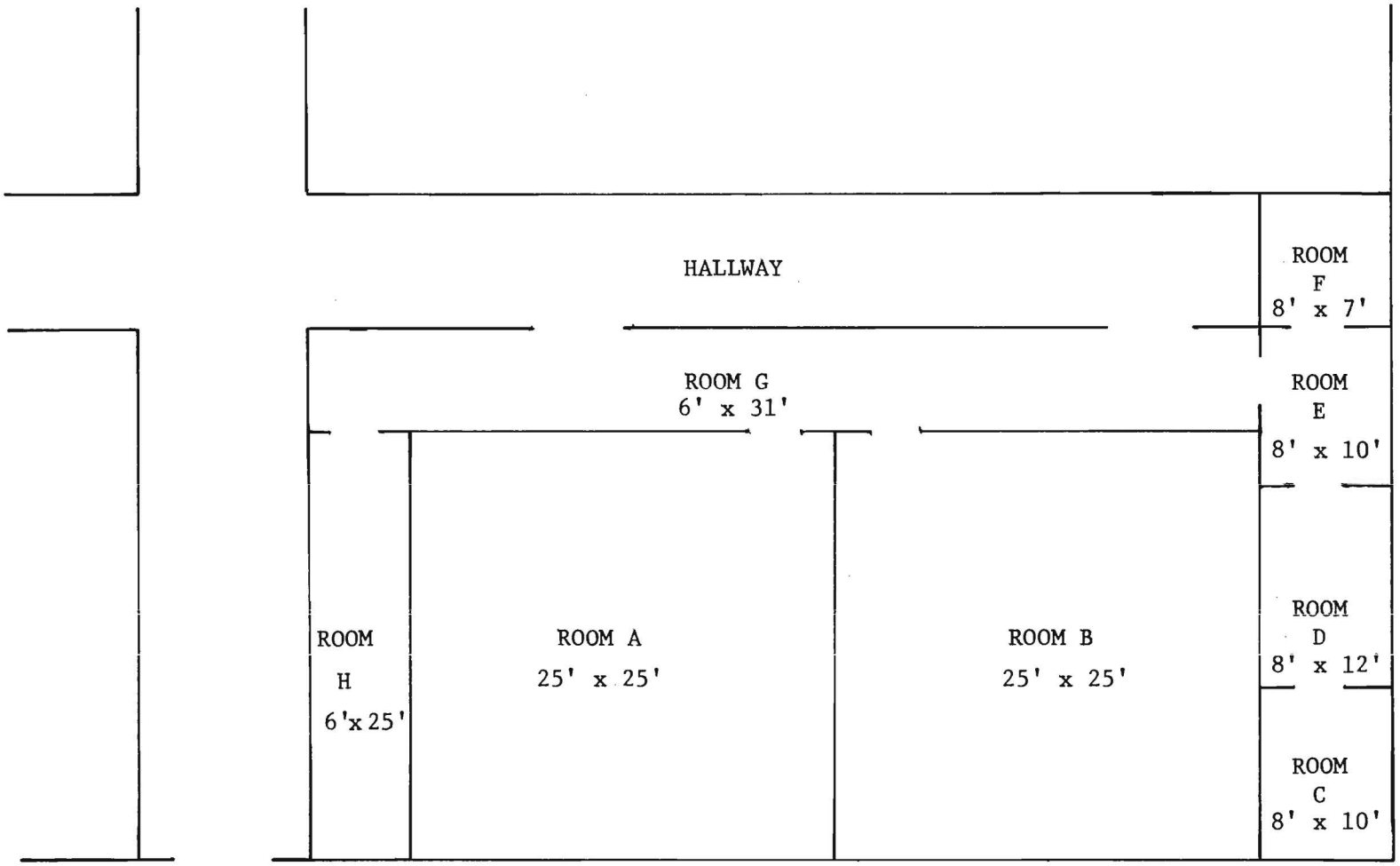


Figure 1. Floor Plan for the Radiofrequency Radiation Facility.

- housing large populations of exposed and sham-exposed rats in two identical and adjoining rooms,
- both storage of suppplies and spare parts, and for maintenance of equipment and cages,
- a commercial cage washer,
- transfer of rats from exposure cages into holding cages,
- weighing the rats,
- drawing microsamples of blood from the rats,
- a buffer zone between the Control/Radiation Rooms and routine activity in the Baker Building, and
- housing and operating the 435-MHz transmitter.

B. Exposure Field Description

Slotted-cylinder antennas were selected to feed the circular, parallel-plate waveguides because they generate a horizontally-polarized field with an essentially-constant amplitude pattern. Performance of these antennas can be intuitively understood by referring to Figure 2, where the antenna is first considered to be a slot in a flat metal sheet [4]. This slot is fed at its center with a coaxial cable and the current directions are indicated by arrows. The sheet metal may then be formed into a U-shape and finally into a cylinder, with the coaxial feed cable located inside the cylinder. If the impedance around the cylinder circumference is sufficiently low, current will flow in horizontal loops around the cylinder. Under these conditions, the slotted-cylinder functions as an antenna radiating a horizontally polarized field, the amplitude of which is dependent on the cylinder diameter. In general, the radiated field tends to be greater on the cylinder side where the slot is located; however, if the cylinder diameter is a sufficiently small part of a wavelength (approximately 0.1λ), the radiated field in the horizontal plane becomes essentially uniform. If the cylinder diameter is increased to the point of becoming a significant part of a wavelength, the field in the region of the shadow cast by the cylinder becomes small. Generally, as the cylinder diameter becomes large, the horizontal field approximates a cardioid [5].

On a previous program [2], the interactive relationship between slot width, slot length, cylinder diameter, and cylinder wall thickness was investi-

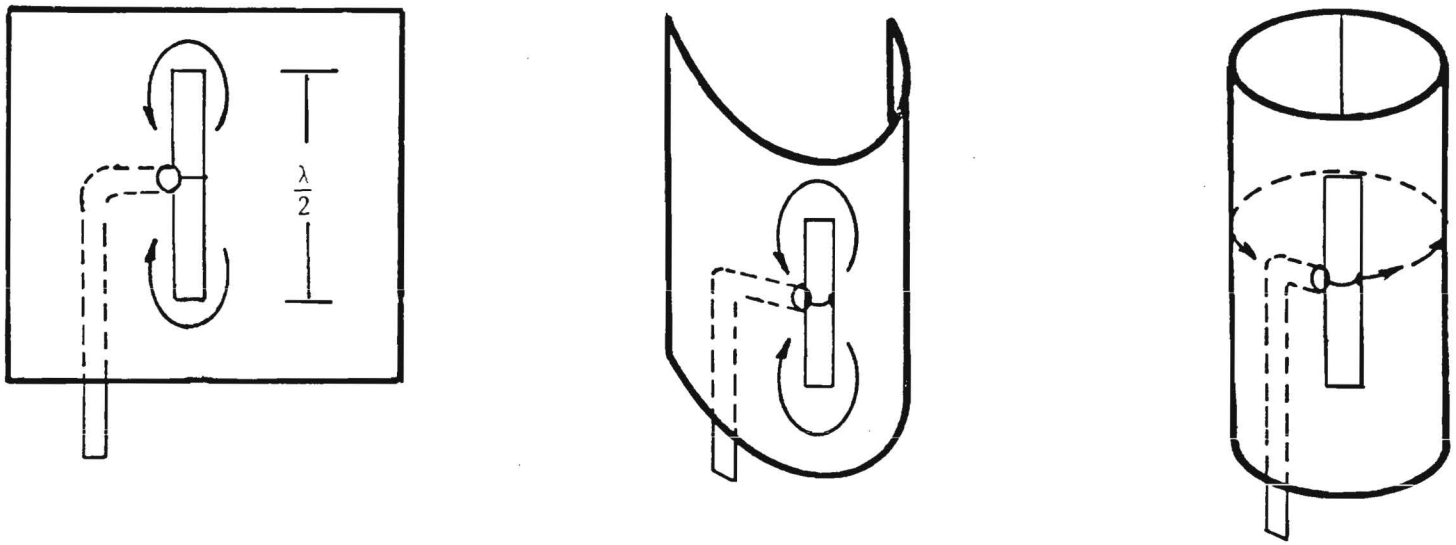


Figure 2. Formation of a slotted-cylinder antenna (see Reference 4).

gated, and it was concluded that a slotted-cylinder antenna with the following features would provide a suitable feed for the circular, parallel-plate waveguides:

- cylinder diameter: 4 inches
- cylinder wall thickness: 0.125 inches
- slot length: 14 inches, and
- slot width: 0.125 inches.

Four sets of circular, parallel-plate waveguides were stacked one above the other to provide a structure for exposing a large population of rats, and an identical structure was provided for housing control rats. The 12-foot diameter of the plates made it possible to position 25 Plexiglas cages around the inside periphery of each set of plates (see Figure 3) while maintaining a cage center-to-cage center spacing of 18 inches. This separation distance resulted in intercage scattering of the exposure field being undetectable with small dipole probe antennas and a sensitive receiver tuned to the exposure frequency. The spacing between individual sets of plates in each structure was 18 inches since, at the 435-MHz exposure frequency, this distance was greater than one-half wavelength, but less than one wavelength. This wavelength relationship assured that only the lowest-order TE-mode propagated outward in concentric circles about the slotted-cylinder feed antennas located at the plate centers. The electric field vector is necessarily zero at the plate surfaces, thereby preventing mutual coupling of fields between adjoining sets of plates. An extensive number of radiation pattern measurements were made with the resulting data confirming TE_{01} propagation in which the vertical component of the electric field vector was typically 17 dB below the horizontal component (see Figure 4) at 435 MHz.

During the shakedown evaluation, the transmitter was connected to slotted-cylinder antennas at levels 2 and 3 of the circular waveguides in the Radiation Room. The remaining two transmitter outputs were connected to dummy loads. The transmitter output was adjusted to provide a 1.0 mW/cm^2 exposure field at a position half way between the plates at levels 2 and 3. This exposure field was measured with a Narda Model Probe every 30 degrees around the plate circumference to determine uniformity of the exposure field. As in previous measurements, the power density varied by less than $\pm$ 1 dB around the plates at both levels. These variations were reconfirmed in measurements

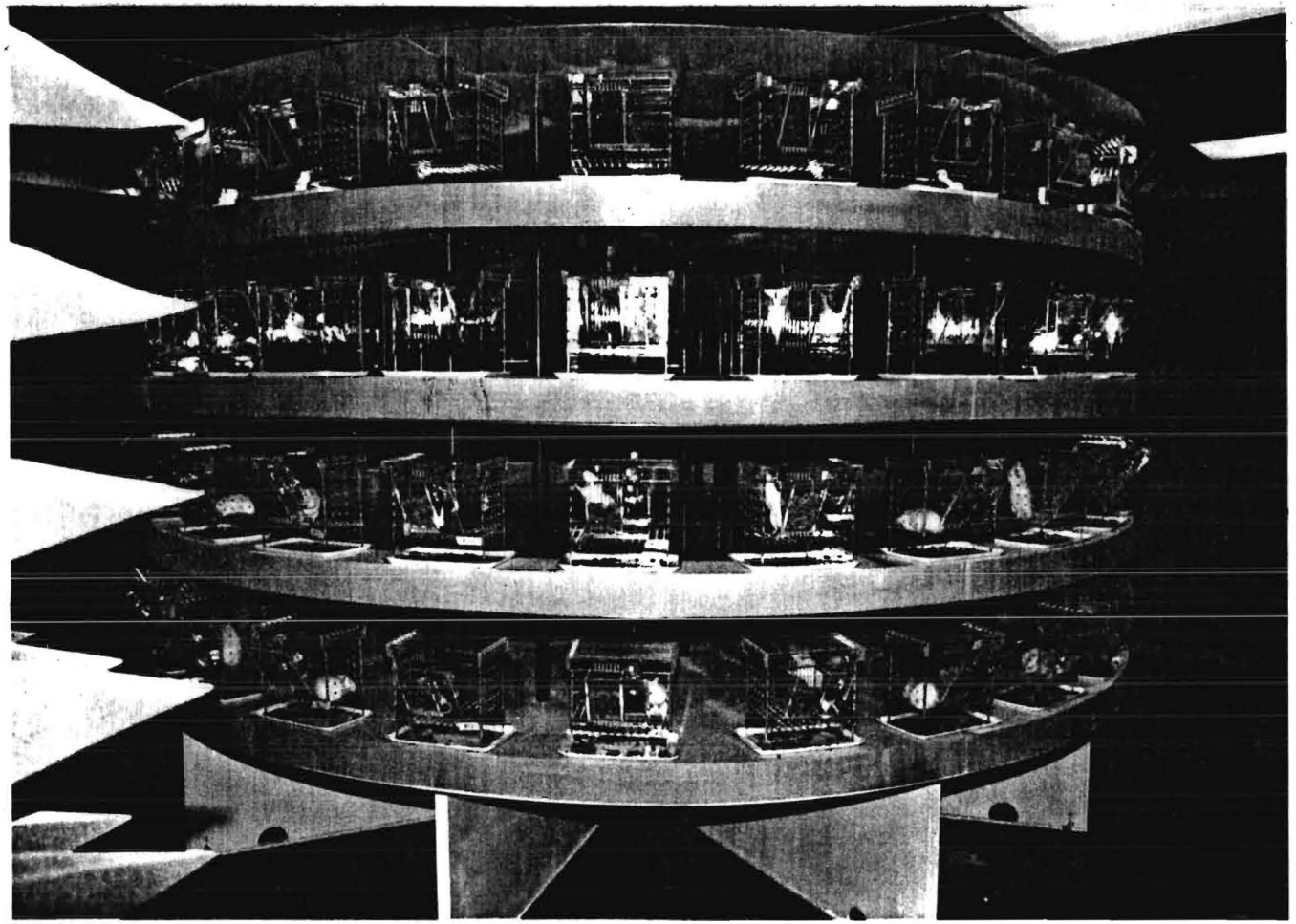


Figure 3. Rats Positioned on Circular, Parallel Plate Waveguides.

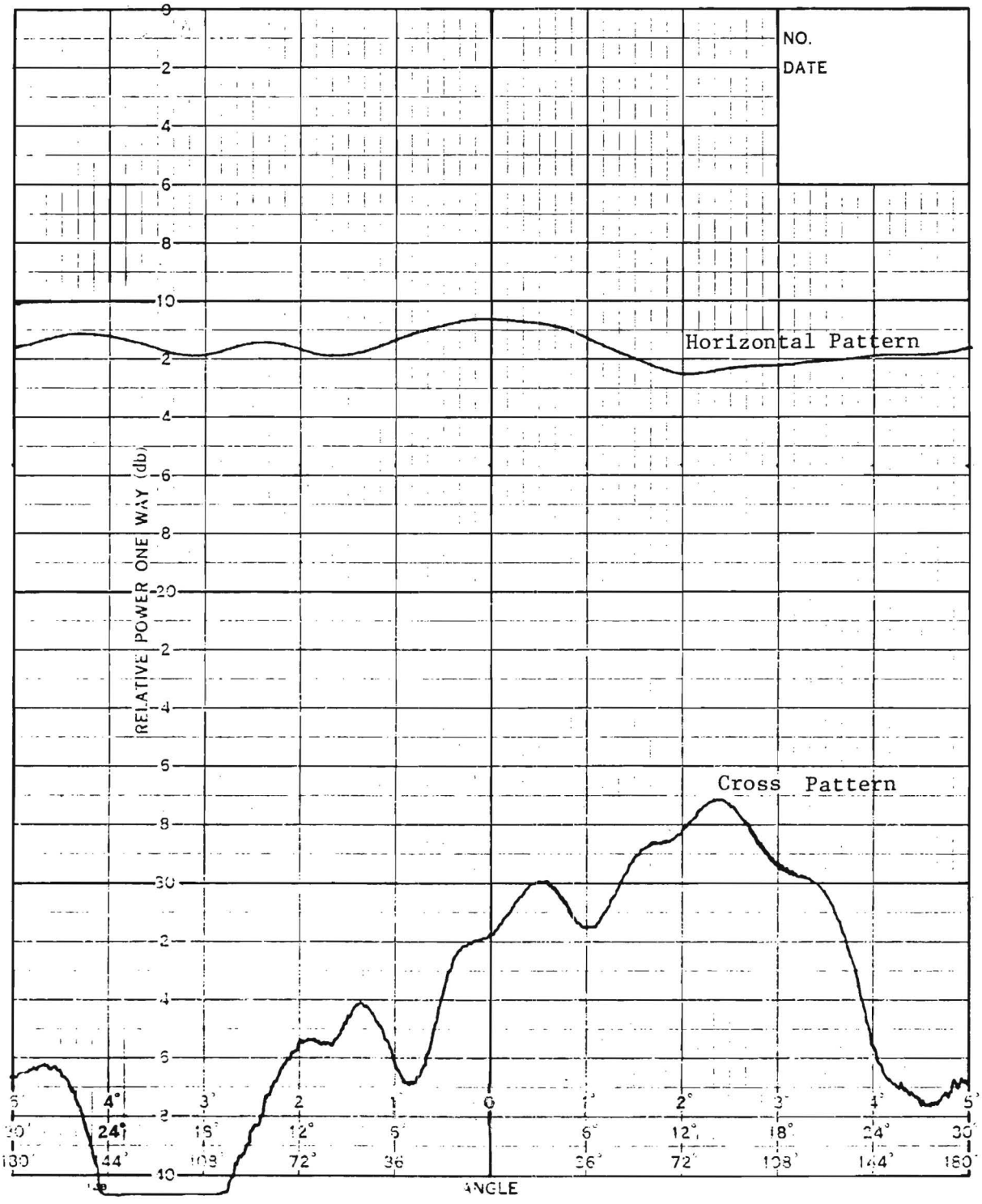


Figure 4. Typical radiation pattern at 440 MHz obtained with prototype, circular, parallel-plate waveguide and slotted-cylinder antenna.

made later with a three-dimensional I-beam electric field probe on loan from the Bureau of Radiological Health [6]. With this probe, the signal processing and display device was located outside the Radiation Room and interconnection with the probe was provided by a fiber optic cable.

When the space provided by eight contiguous rooms is considered, along with independent heating, air conditioning, lighting, and water supply systems plus open-ended waveguides for exposing/sham-exposing experimental animals, it is seen that the RFR Facility provides an essentially self-contained area for conducting a wide variety of either long-term, low-level, or short-term, high-level bioeffects studies.

SECTION III
DATA ACQUISITION SYSTEM

In developing overall performance capabilities for the data acquisition system, it was noted that the system must (1) automate the data logging procedure to the maximum extent possible, (2) monitor the status of the exposure fields, (3) provide a home alert in case the exposure fields drop below predetermined levels during non-work hours, (4) perform basic statistical analysis of the data, and (5) interface with the Georgia Tech large mainframe computers for more extensive data processing. In conversations with AFSAM personnel, it was also agreed that the system should be compatible with those used in chronic bioeffects studies at AFSAM and the University of Washington. Compatibility with the AFSAM and University of Washington systems dictated a system configured around the industry standard S-100 bus. This bus standard was originally known as the "Altair" bus appearing in the MITS Altair computers in 1975. Because of its tremendous design flexibility, it was quickly adopted by many microcomputer manufacturers and is now regarded as the most-used busing standard ever developed by the computer industry. Physically, it consists of a set of 100-contact edge connectors mounted to a common motherboard and wired in parallel. Modules plugging into the edge connectors are circuit boards measuring 5 x 10 inches.

With the above system requirements in mind, a review of commercially-available, S-100 compatible computers was conducted, with the results that the Northstar Horizon and Cromemco System Two units were determined acceptable. Both offered a system core consisting of S-100 motherboard, Z-80 8-bit processor chip as the Central Processing Unit (CPU), 64 K Random Access Memory (RAM), two drives for 5.25-inch, double-sided, dual-density disks, a controller, a cabinet, and necessary power supplies. Retail prices for the Northstar Horizon and Cromemco System Two units were $4,330 and $4,695, respectively; however, both systems were available at discounts of approximately $750 from several different computer marketing centers. As capabilities of the two systems were compared in detail, it was noted that either could be used for this program, but that the Cromemco System Two offered 390 kbytes per drive and a motherboard with 21 board slots. Comparable features of the

Northstar Horizon system were 380 kbytes per drive and 12 board slots. Based on these differences in technical specifications, the Cromemco System Two unit was purchased and the system configuration shown in Figures 5 and 6 was developed.

In describing the data acquisition system shown in Figure 5, it is first noted that the S-100 bus was originally designed for use with a CPU based on the 8080 microprocessor; however, in the Cromemco System Two, the CPU has been designed around the Z-80 microprocessor. Cromemco's designation for this Z-80 based CPU is ZPU. The ZPU in Figure 5 has the following technical specifications:

Processor: 4 MHz version of the Z-80 microprocessor

Clock Rate: 2 or 4 MHz (switch selectable)

Instruction Set: 158 instructions including the 78 instructions of the 8080 microprocessor

Power On Jump: Jumper-wire enabled

Power-On Jump Locations: 16 switch-selectable locations

Wait-State Generations: 0-4 jumper-selectable wait states

M1 Wait State: Jumper wire selectable

Bus Compatibility: S-100

Power Requirements: + 8 VDC at 1.1A

The S-100 bus interfaces the Z-80 CPU module to as many as 20 additional memory, input/output (I/O) or other processor modules. Signals associated with this bus can be grouped into four categories as follows: (1) power supply, (2) address, (3) data, and (4) clock and control. The power supply signals involve three unregulated DC voltages as follows: +8, +18, and -18 volts. Since the main power supplies are unregulated, power supply regulation must be provided on individual circuit cards. For the address signals, there are 16 address lines that allow direct addressing of 65,536 words of memory space. Tri-state TTL drivers are used to drive the address bus. To handle data signals, the S-100 bus has two directional data buses, each eight bits wide. All clock and control signals are standard TTL levels and there are three clock signals on the S-100 bus.

The 64 kbyte RAM is provided by a S-100 bus-compatible read/write memory board with the following specifications:

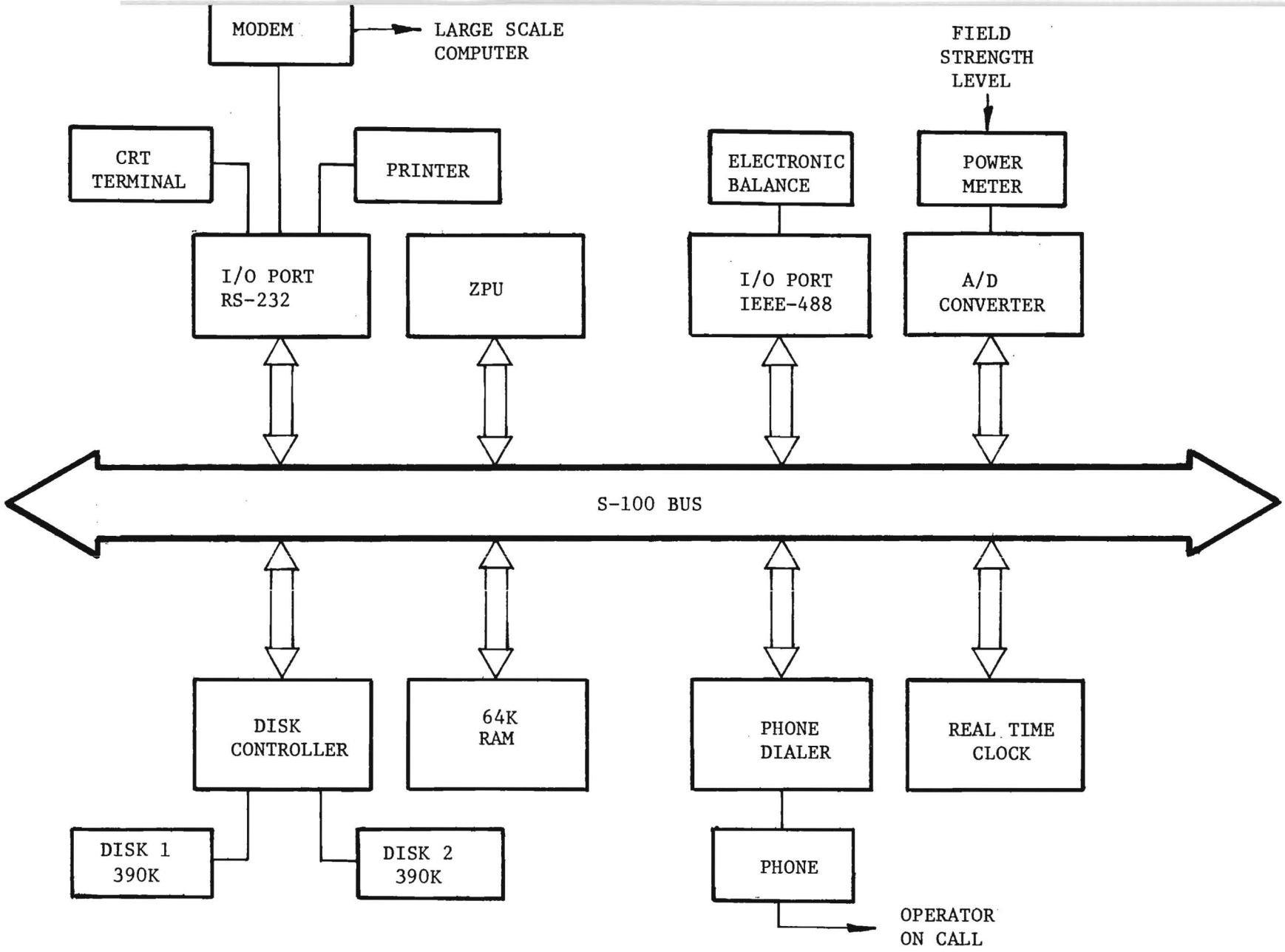


Figure 5. System Configuration for Cromemco System Two Data Acquisition System.

Figure 6. Photograph of Data Acquisition System.

Memory Capacity: 65,536 Bytes

Memory Type: TMS 1416-15, 16K X 1 dynamic RAM

Memory Access Time: 250 nanoseconds (max)

Wait States at 2 MHz: None required

Wait States at 4 MHz: None required

Bus Compatibility: S-100

Power Requirements: +8, +18, and -18 volts

Interfacing of the CRT terminal, modem (for communicating with Georgia Tech mainframe computers), and printer with the S-100 bus is provided by a Cromemco D+7A Input/Output Module. Specifications for this module are as follows:

Analog Input Ports

- Number: 7
- Input Voltage Range: -2.56 to +2.54
- Resolution: 8 bits
- Conversion Time: 5.5 microseconds

Analog Output Ports

- Number: 7
- Output Voltage Range: -2.56 to +2.54
- Resolution: 8 bits
- Conversion Time: 5.5 microseconds

Parallel I/O Port

- Input Port: 8 bits
- Output Port: 8 bits
- Input Load: one TTL equivalent
- Output Drive: 10 TTL loads

Bus Compatibility: S-100

Power Requirements: +8, +18, -18 volts

A Pickles and Trout Model P&T-488 interface permits the electronic balance to communicate with the S-100 bus. This interface appears as four I/O ports that are addressed as consecutive ports with the first port address an integer multiple of 4 (0,4,8,...). These ports allow the ZPU to manipulate the data, handshake, and bus management lines of the IEEE-488 bus.

The clock-calendar card shown in Figure 5 is a Scitronics, Inc. Model RTC-100 unit and is used to provide the timing signals necessary for monitoring field strength in the Radiation Room on an interrupt-driven basis. This

monitoring function also requires an A/D converter to digitize the output of the power meter and communicate it to the S-100 bus.

In order to provide a hard copy of the data, the data acquisition system includes a Microline Model 82A Printer. This unit employs an impact dot matrix print system in which characters are a 9 x 7 matrix of dots. The printing direction is bidirectional and the printing speed is 120 characters per second with the normal character spacings of 10 and 16.5 characters per inch. The unit prints alphanumeric characters and symbols plus lower-case English letters and symbols. A tractor unit is provided to feed paper into the printer. Interfacing of the printer with the S-100 bus is via a low-speed serial port based on the RS-232 code.

SECTION IV

CAGE WASHER SYSTEM

After reviewing the technical specifications, cost, size, etc., of cage washers from several manufacturers, the Southern Cross Model 900-A Dyna Jet Washer was purchased and installed in Room D of the RFR Facility as shown in Figure 7. This washer is of stainless steel construction and provides fully automatic wash, rinse, and final rinse cycles for all types of animal cages and accessories. A tempered safety glass viewing window is installed in the washer front. Loading of the washer is accomplished from the left (soil) side through a guillotine, pass-through door with a safety interlock feature that prevents washer operation until the door is closed. Exit is on the right (clean) side through a pass-through door identical to the one on the entry side. Cage washing is provided by water jets from motor-driven, rotating, stainless steel manifolds located above and below the wash compartment. A 35-gallon detergent tank is located below the wash compartment and used for the wash cycle. This tank contains a heavy-duty heating element with an external control so wash water temperature can be varied from room temperature up to 190°F. The rinse cycle uses hot tap water from the building utility supply. For final rinse, a 27-gallon tank with an automatic water level controller is provided. External plumbing provides for the introduction of special final rinse fluids (distilled water, dionized water, etc.) and disinfectants.

Automatic reset timers control the wash and rinse time intervals as follows:

Wash Cycle: 0 to 10 minutes

Rinse Cycle: 0 to 10 minutes

Final Rinse Cycle: 0 to 1 minute.

These timers are wired such that any cycle can be omitted by adjusting the cycle timer to the "off" position. Once the timers have been set to the desired cycle durations, the start button is pushed and the wash operation is automatically continued through all cycles.

To facilitate wash and rinse operations, special-purpose stainless steel racks for the Plexiglas cages and glass water bottles are provided.

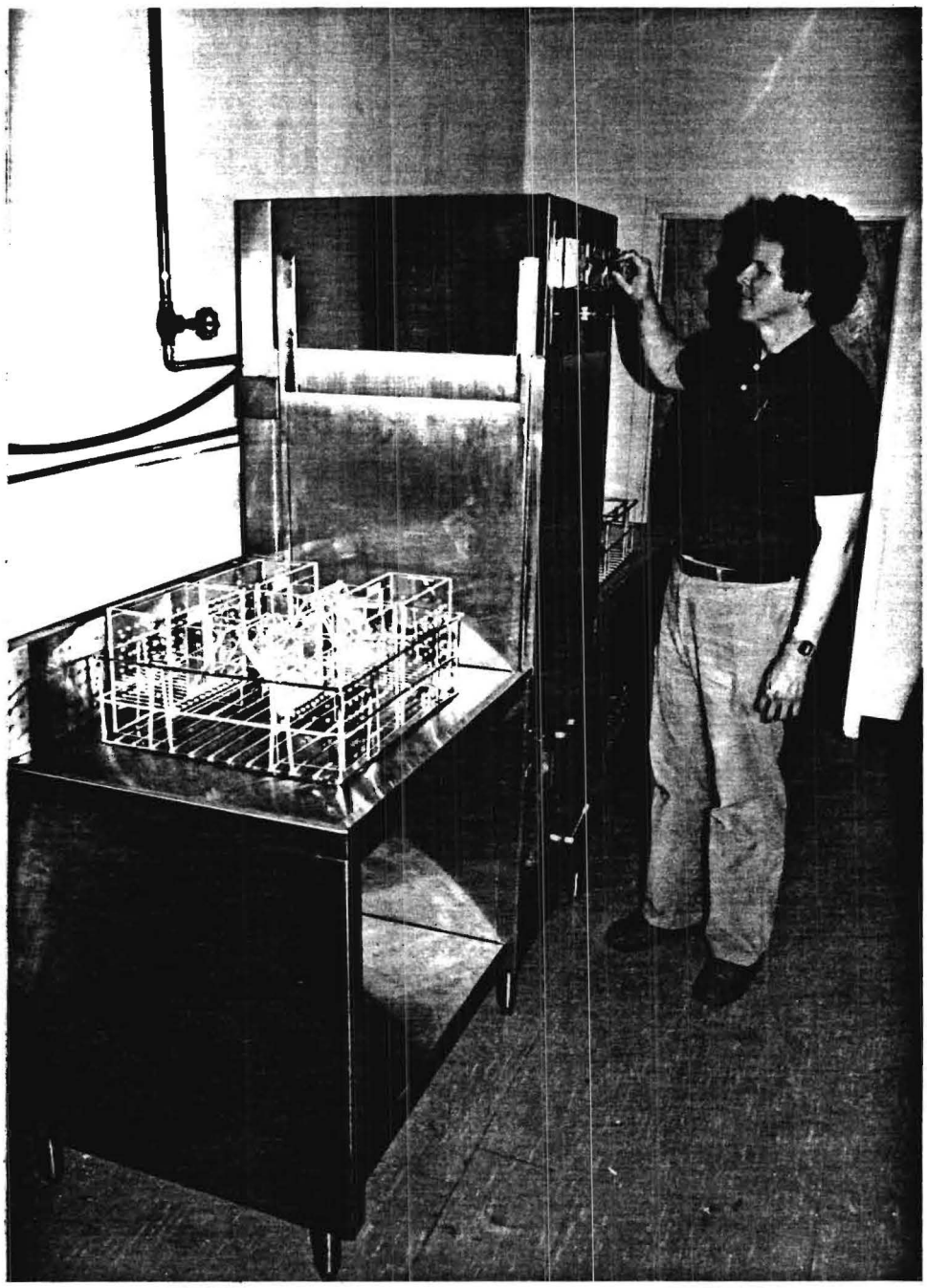


Figure 7. Photograph of Cage Washer.

These racks hold four cages and 49 bottles, respectively, in the wash compartment of the washer. The bottle rack provides a cover of stainless steel mesh to prevent water jets in the washer bottom from forcing bottles out of the rack during wash and rinse cycles.

SECTION V

ELECTRONIC BALANCE SYSTEM

Another important system purchased and installed during this program was the electronic balance. After reviewing technical capabilities of balances from several manufacturers, the Sartorius Model 1203 MP Balance with built-in microprocessor, variable integration time, and locked-in readout was purcahsed. This balance offered a weighing range and readibility of 0 to 4000 grams and 0.1 gram, respectively. In addition to the balance, a Sartorius Model 704201 Keyboard Programmer and Model 705306 Cable box were also purchased. The resulting electronic balance system was used to accurately weigh excited rats and automatically store the weight data in the data acquisition system. The keyboard programmer made it possible to program the balance for a variety of different weighing procedures including net weight, mean weight, average weight, accumulated weight, and difference from a predetermined average weight. The weight displayed by the balance was actually the average of a programmed number of individual weighings performed over either a fast (0.5 seconds), normal (1 second), or slow (2 seconds) integration time. Once the programmed number of weighings was completed, the average weight was locked in and displayed without fluctation on a large, seven-segment digital readout. The taring capability of the balance was instantaneous and was accomplished by touching the tare switch on the balance front. When the weight data displayed on the readout had achieved stability, a small "g" symbol appeared next to the weight value and indicated accurate data.

In most instances during the shakedown evaluation, the balance system was not interfaced with the data acquisition system because the wrong interface module was ordered. Late in the shakedown evaluation, the correct interface module was received and the following program was used to accurately weigh the rats:

KEY	FUNCTION
g	Tare the balance Calls up stored regular weighing program
	Select weighing range on balance (0-400 grams)

10	Key-in the desired number of individual weighing (in this case, 10)
CS	Store the desired number of individual weighings
25	Key-in rat identification number (in this case, number 25)
#	Transfer the number 25 to data output
	Place rat on balance pan
A	Start program. The balance automatically weighs Rat # 25 ten times, displays the number of individual weighing, stores and adds up the individual weight values, calculates the mean weight, displays the mean weight, and transfers the mean weight value to the data output.
	Tare the balance
	Place next rat on the balance pan
A	Start program

SECTION VI
EXPERIMENTAL ANIMALS

All exposed and sham-exposed animals were carriers of chronic aortic cannulae. The distal end of the cannula was positioned in the aortic arch (only 1-2 mm) while the proximal end was exteriorized at the back of the neck. Implantation of the cannula was done under nembutal anesthesia. Once implanted, the cannulae remain patent for months, some for the rat's lifespan [7-8]. Over 16,000 rats have now been cannulated in Dr. Popovic's laboratory. Adverse effects of cannulation of the aorta have never been described, nor has published criticism of the procedure been offered.

During this program, 150 Spraque-Dawley rats were used. The rats were delivered from the breeding colony (Harlan Sparque-Dawley, Madison, WI) to Dr. Popovic's animal quarters at Emory University. The animal quarters are fully isolated, being private quarters to Dr. Popovic's laboratory. A rigid schedule of visual observation, care, maintenance, and cleanliness is enforced, resulting in ultraclean animal quarters and research laboratories. The lighting cycle in these quarters was 8 am to 8 pm and the temperature was maintained at 24 $\pm$ 1^{o}C. In these animal quarters, the rats were provided food (Purina Rat Chow) and water ad libitum and carefully screened for health problems while growing to a body weight of 170 to 180 g. While growing to this weight, health status was monitored daily, and body weight was measured twice weekly. Rats that did not follow normal body weight curves (established for this strain during years in Dr. Popovic's laboratory) were eliminated as were those with an increased total white blood cell count or an unusual differential count (0.3 ml of arterial blood sampled once per week).

When the rats reached a body weight of approximately 175 g, 75 of them were randomly selected for cannulation (all animals were cannulated by the same person, biomedical technologist C. Honeycutt, MSc). The cannulated rats were returned for seven days to their individual cages in the animal quarters for recovery from the cannulation. Fifty cannulated rats, in addition 50 others that were not cannulated, were randomly selected and then transferred to the RFR Facility at Georgia Tech where daily visual observations continued and the rats were weighed twice weekly. In the RFR Facility, the rats were

housed in Plexiglas cages specially designed for this program and positioned on the circular, parallel-plate waveguides in the Control and Radiation Rooms, where their acclimation to the new environment took place. A laboratory record was opened for each rat, and both environmental factors and health activity were placed in the animal's record. Neither the biomedical technologist who drew blood samples nor the assay personnel who analyzed the blood samples were able to distinguish between exposed and sham-exposed rats. Baseline data were obtained from the blood samples drawn and assayed for the biologoical endpoints proposed earlier.

SECTION VII

SHAKEDOWN EVALUATION

The shakedown evaluation was conducted to assure that the numerous tasks associated with (1) cannulating and managing a large population of rats, (2) operating the exposure facility, (3) drawing microsamples of blood, and (4) assaying the blood samples could be satisfactorily accomplished. The evaluation was conducted over an eight-week period and is described in the following paragraphs in terms of the biological endpoints monitored, the experimental procedures used, and the results obtained.

A. Biological Endpoints

The effects of long-term exposure to low-level RFR on rats and other mammals was discussed in Georgia Tech Proposal No. EC-BR-1216, "Long-Term, Low-Level Bioeffectts Study Using 200 Cannulated Rats Exposed to 453-MHz Radiofrequency Radiation," pages 7-11. This discussion concluded that, with a few exceptions in which artifacts were suspected, long-term exposure to low-level RFR does not appreciably alter biological parameters. This, of course, does not necessarily mean that all of the parameters stay unchanged; it is possible that some more sensitive or more reactive endpoints do change. It is also possible that, after changing (which indicates a specific physiological distrubance), the endpoints revert back to normal or near-normal levels because of adaption on the part of the biological system. An example of endpoints likely to exhibit this behavior is hormones. The rationale for monitoring hormones is that exposure to RFR represents an environmental condition that might be stressful to biological systems. Certain humoral changes are known to be sensitive responders to stress. Of course, to detect the early beginning of such humoral change would be of interest because of the crucial role of hormones in providing physiologic control of all vital processes and because it would provide an early clinical test to detect any possible harmful effect of RFR. The inter-relationship among hormones provides both the flexibility and small gradations in response that are fundamental to homeostatic control. Hormones play a crucial integrative and regulatory role in major processes including energy production, control of body fluids, growth, development, and others. Control of hormone secretion

is especially important in maintaining homeostasis. Such control is achieved by a negative feedback mechanism in which the hormone producing a biological effect achieves a sufficient magnitude to inhibit its further secretion. This negative feedback system consists of multiple components involving detection of a real or threatened homeostatic imbalance, a means of signaling secretion from secretory cells, responder organs or organ systems, and shutoff of secretion when adequate hormone levels are reached. By these mechanisms, the delicate physiologic balance required for homeostasis is maintained.

For this study, adenohypophyseal adrenocorticotropic hormone (ACTH), adrenal corticosteroid cortisosterone, catecholamines epinephrine and norepinephrine, growth hormone and prolactin were chosen as biological endpoints. Selection of these hormones was based on their common link to the existence of exogeneous stressors. This common link is evident when literature dealing with possible effects of environmental stress on plasma levels of these hormones, and possible mechanisms that might be involved, is reviewed. Furthermore, some of the results obtained in stressed animals, and the importance of determining basal hormone levels for correct evaluation of the imposed stress, are also evident. In the following paragraphs, some of the more significant research findings in this area are summarized.

It is generally accepted that catecholamines, ACTH, corticosterone, growth hormone and prolactin are good indicators of stress. This is true for man and it is true for many animals, especially for rats - a species studied more than any other. The concept of stress has been widely accepted as a specific somatic response to damage, or threat of damage, by a wide variety of environmental agents. This concept was first suggested by the observation made in 1911 by Cannon and de la Paz. They showed that the adrenal medulla releases hormones in the cat during the emotional excitment associated with exposure to a barking dog [10]. In 1936, Hans Selye demonstrated evidence of a second endocrine system, the pituitary-adrenal-cortical axis [11]. This system, responding often to a more subtle psychic and physical stress, was able to induce a more global and profound influence on metabolic functions.

Other endocrine systems (in addition to those involving the adrenals) can respond to stimuli also. In light of recent work, it seems that no endocrine system is entirely free from the influences of stress. Despite

these recent advances implicating virtually all endocrine systems, altered pituitary and adrenal function in relationship to the handling of stress has remained the central focus for present research.

In this respect, rats have been studied more often than any other animal species, as already mentioned. The literature showed that mostly Sprague-Dawley rats were used, with Wistar rats used in a few cases. In most investigations, the rats were sacrificed by decapitation before and after stress, plasma was collected, and then assayed on the hormonal level. In a very few investigations, retro-orbital venous plexus punctures were used [12]. Blood sampling after cutting the tail was used more often [13]. In a somewhat larger number of investigations, the rats were bearers of venous catheters (PE 50, 51, 60, 61, 62) and were used a few hours or, at the most, one or two days after cannulation [10, 40]. Carotid artery cannulae were used more often [14-19] than venous cannulae, but also only a few hours (at most, a day or so) after cannulation. It is believed that sampling blood from semi-chronic cannulae does not affect basal level of hormones; however, there are no studies to show when animals have fully recovered from anesthesia and surgery. In one study [15], the level of catecholamines was found to be the same in arterial and in venous blood. Repeated collection of 0.5-ml blood samples three times in 30 minutes equaling 1.5 ml, or nine times equaling 4.5 ml in 12 hours, did not change the values of basal catecholamines [14], a finding difficult to accept. It has been shown that a decreased blood volume increases catecholamine levels in plasma [20,21]. Some researchers used fresh, and some frozen, blood [22,23]. The plasma levels of "stress hormones" showed a clear circadian (approximately 24 hours) rhythm [11,12]. Circadian increases of plasma corticosterone [24,25] were large, with perhaps a six-fold difference observed between the lowest (early morning) and highest (late afternoon or early evening) values [26]. The plasma hormonal levels were increased after stress [27-31], but plasma growth hormone level in the rat was decreased after stress [26,32,33]. Some hormones, for instance ACTH, increased much after stress, sometimes ten-fold, but other hormones increased only slightly as, for instance, corticosterone [34]. Plasma prolactin was already increased two minutes after a mild stress [35]. It is generally accepted [36] that some hormones (for instance, catecholamines) respond to increasing magnitudes of stress in a step-wise fashion (i.e., respond

monotomically), while others (for instance, corticosterone and prolactin) respond in an all-or-none fashion [27], but not all investigators agree with the second part of this statement. Some investigators felt that plasma corticosterone increased with increasing levels of stress in a step-wise fashion [37], just as catecholamines do. Hennessy and Levine were able to prove that "corticoid levels can sensitively reflect differences in the intensity of stimulation" [38]. The response of the pituitary-adrenal axis to stress [27-31] was striking. After short stress, the increased plasma level of hormones had a duration of about 30 minutes [39] or less, depending on the strength and duration of stress. Some investigations suggested that rats which had not been subjected to daily handling showed a more dramatic stress-increased serum prolactin level [27], and that training (periodic handling) lowered the level of "stress hormones" [14], but there are other reports that handling (for four days in male rats) might increase prolactin response to stress [40]. For corticosterone, the biggest increase resulted from merely placing the rat in the test chamber [22]. Blood sampling in one rat affected the level of plasma "stress hormones" of other rats in the same room. Immobilization increased plasma catecholamines and corticosterone in conscious rats [17,37,41-46]. Stress increased plasma ACTH [47] and lead to pituitary-adrenal activation [48]. Even after a two-hour immobilization, the plasma hormone values were still at the same high level as in the early beginning of the stress [49]. Repeated stress decreased reaction of "stress hormones" [50] causing "habituation". Values of plasma catecholamines and plasma prolactin were higher after decapitation than when blood was sampled from catheters [45,47]. Plasma growth hormone level, like adrenal steroids, has been found to be very responsive to stress [51-53], demonstrating a rapid fall after the stress. It seems likely that growth hormone has circadian rhythmicity [51] which is inversely correlated with diurnal levels of corticosternone [23] and other homrones. However, not all investigators agree with the existance of circadian rhythm in growth hormone release [25,54] because of the inherent large variability of the data. Growth hormone (and other stress hormones) is released in an episodic fashion in resting (nonstressed) rats. Thus, mean growth hormone levels exhibited characteristically high variability [55]. Besides the studied hormones, plasma renin activity [56] and gonadotrophins might also be useful indicators of induced stress. Of

course, catecholamines are important controllers of renin release [57] and thus, catecholamine determination without renin activity measurements might be sufficient.

B. Experimental Procedures

During this program, experimental procedures were developed for monitoring hormone levels in a large population of rats exposed to pulsed-wave RFR at a frequency of 435 MHz. The hormone levels were monitored by assaying microsamples of blood drawn from the rats via their cannulae. The studied hormones respond to an exogenous stress in a manner similar to the dose-response relationship, i.e., the larger the stress, the greater the change in the plasma of stress hormones. Further, to study changes in ACTH, corticosterone, and prolactin, it is sufficient to draw only 0.3 ml of arterial blood from the chronic aortic cannula. There is no wasting of blood, a very small amount of blood is drawn weekly, samples consist of adequately mixed blood, the same rat can be used as its own control, and the rats can be followed before, during, and after RFR exposure lasting weeks or months.

On Monday, 26 April 1982, the rats were received at the Georgia Tech RFR Facility and began a two-week acclimation period. During this time, the temperature and humidity in the Radiation and Control Rooms were continuously monitored and recorded using calibrated meters provided by the Physical Plant. A typical chart from one of the recorders is presented in Figure 8 and shows that temperature and humidity were maintained at $72 \pm 2^{\circ}F$ and 50 ± 2 percent, respectively. During this two-week period, rats from the exposure group were handled (placed in the small Plexiglas boxes) numerous times as part of their habituation process.

Two weeks after receipt at Georgia Tech, the rats were considered to have completed their acclimation period and the shakedown evaluation with RFR began. Microsamples of blood were drawn from the cannulated rats on this day, and on each of the following five Mondays, between the hours of 10 am and 2 pm. During these hours, the plasma levels of the measured hormones are at their lowest values. The experimental procedure involved de-energizing the 435-MHz pulsed-wave (pulse rate = 1000 pps, pulse rate = 1.0 microsecond, exposure power density = 1 mW/cm^2) transmitter while a member of the program staff (animal caretaker) entered the Radiation Room with a cart designed to accommodate eight Plexiglas cages. (It is noted that this staff member

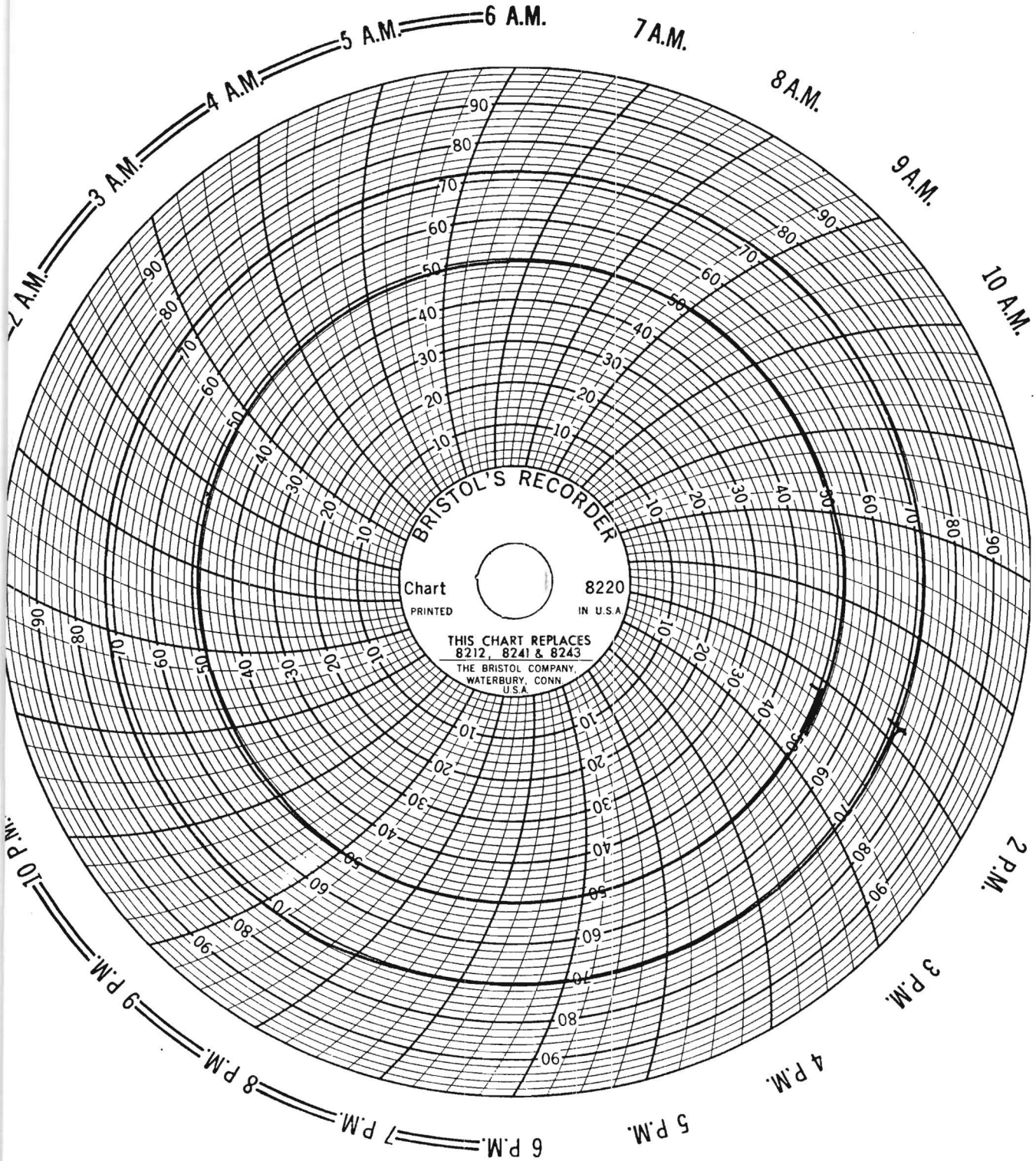


Figure 8. Chart Showing Temperature and Humidity in the RFR Facility.

was the only person authorized to work with the rats during procedures that involved the drawing of blood samples). Eight numbered cages were removed from the circular, parallel-plate waveguides, placed on the cart, and transferred to Room E, Figure 1. Once the staff member and rats were out of the Radiation Room, the transmitter was energized again. In Room E, the eight rats were transferred to small, black, Plexiglas boxes just large enough to house an individual rat and provide a comfortable space large enough that the rat can turn around but doesn't struggle to escape. The Plexiglas tops for these boxes were slotted so the cannulae could extend exterior to the box and be accessible for blood sampling. These small Plexiglas boxes were pre-numbered to correspond to the numbers on the larger Plexiglas cages used to house the rats while in the Radiation Room. Placement of the rats in the small Plexiglas boxes was done with slow, deliberate movements and in a manner that caused a minimum excitement of the rats. When all eight rats had been transferred to the small Plexiglas boxes, they were then moved to Room F, where a settling down period of at least 15 minutes began. Once the settling down period of approximately 15 minutes had passed, the rats appeared to rest comfortably (sleep, in most cases) and blood samples could be drawn during this resting period. Studies conducted during earlier programs have shown that this resting period extends for approximately four hours before the rats become restless. During this period, (and the following period during which blood samples were drawn), only the biomedical technologist was permitted in Room F. Also, the door to the room was closed to prevent sounds from other areas within the Facility from exciting the resting rats. After 15 or more minutes, a 0.3-ml blood sample was drawn with the cannula protruding through the slot in the box top. Mosquito hemostats padded with PE 240 plastic tubing were then used to clamp the cannula slightly below its heat-sealed tip. These hemostats were used to open and close the cannula, as desired, during the remainder of the procedure. The heat-sealed tip was snipped off and the animal's blood pressure slowly forced the heparinized saline solution and blood from the cannula. The heparinized saline solution (0.5 cc heparin--1000 units/ml from beef lung -- per 30 ml of saline solution) was used to fill the cannulae at the time of their implantation. When the heparinized saline solution cleared the cannula tip, a 30 guage needle attached to a 1 cc tuberculin syringe was used to draw 0.3 ml of undiluted blood.

The drawn blood was then transferred to Sarstedt capillary collection tubes calibrated for a 0.3 ml volume and treated with EDTAK to prevent clotting. These tubes were placed in a collection rack submerged in crushed ice where they were maintained until transfer to the assay laboratory. A different syringe and needle were then used to refill the cannula with heparinized saline solution and heat was applied to seal the tip.

A new needle and syringe were used to draw blood from each rat. Also, a new needle was used to refill the cannula with heparinized saline solution after each blood drawing.

This procedure was repeated for each of the eight rats. The biomedical technologist then transferred the rats back to Room E. While blood was being drawn from the first eight rats, a second group of eight rats was removed from the Radiation Room, transferred to small, black Plexiglas boxes, and were ready to begin their settling down period in Room F. As blood was drawn from this second group of rats, the first group was weighed using the electronic balance in Room E. The weight data were entered in the data acquisition system described in Section V. After being weighed, the first group of rats was placed in clean Plexiglas cages and carefully returned to their marked positions on the waveguides in the Radiation Room, and a third group of eight rats was transferred to Room E, in preparation for having their blood samples drawn.

By repeating the above procedure, blood samples were drawn six times during the seven-week evaluation period for the RFR-exposed rats. Blood samples were not drawn during the fourth week because the biomedical technologist was ill. Weight data were taken seven times from all cannulated rats. Other activities associated with the rats involved changing the Styrofoam soil trays under all cages every other day. Food hoppers and water bottles were checked daily, with food and/or water added as necessary. On Wednesday of each week, all cages were washed using the washer described in Section IV. All water bottles were washed on Friday of each week.

C. Experimental Results

Experimental results are presented as plots showing the variations in plasma ACTH, corticosterone, and prolactin for RFR exposed and sham-exposed rats during the shakedown evaluation. Variations in growth hormone and catecholamines are not presented because, at the time blood samples were

available, the specialized equipment needed for assaying plasma catecholamines and the specialized NIH kits needed for assaying growth hormone were not available. Since then, the equipment and kits have been obtained and they are available for follow-on studies.

Variations (mean values plus standard errors of the mean) in ACTH, corticosterone, and prolactin are presented as a function of time for exposed and sham-exposed rats in Figures 9, 10, and 11, respectively.

Sham-Exposed Animals. These animals were used primarily to determine:

- the number of times the animals should be handled in order to reach the true resting level at which the lowest plasma hormone values were reached. The literature, as mentioned earlier, strongly suggests that placing rats into new cages (boxes) induces rather high stress levels. The sham-exposed animals were therefore used during this study to determine the length of the habituation period (the number of times the rats should be handled and placed in the Plexiglas boxes used for blood drawing) necessary for our animals.
- whether the resting level of plasma hormones in our animals was comparable to levels measured by other investigations. This was of special interest because the Plexiglas cages used to house our animals were of a specialized design with a bottom made of glass rods spaced 0.5 inches apart. It was realized that, while such a rod separation would be helpful in maintaining cage cleanliness, it might have forced the animals to exert additional efforts in keeping their balance.

Results of these efforts indicate that, in the case of stress hormones, the first introduction to the Plexiglas box increased the plasma level of ACTH to that representing a mild stress. Plasma levels of corticosterone and prolactin were increased more. It took two (corticosterone) or three (ACTH and prolatin) handlings of our animals before they reached true hormonal resting levels (Figures 9, 10, and 11).

Exposed Animals. During the two-week habituation period in new cages and new surroundings at the Georgia Tech RFR Facility, the rats (prior to RFR exposure) were placed twice in the Plexiglas holding boxes and left there approximately 20 minutes each time. The Week 0 data (Figures 9, 10,

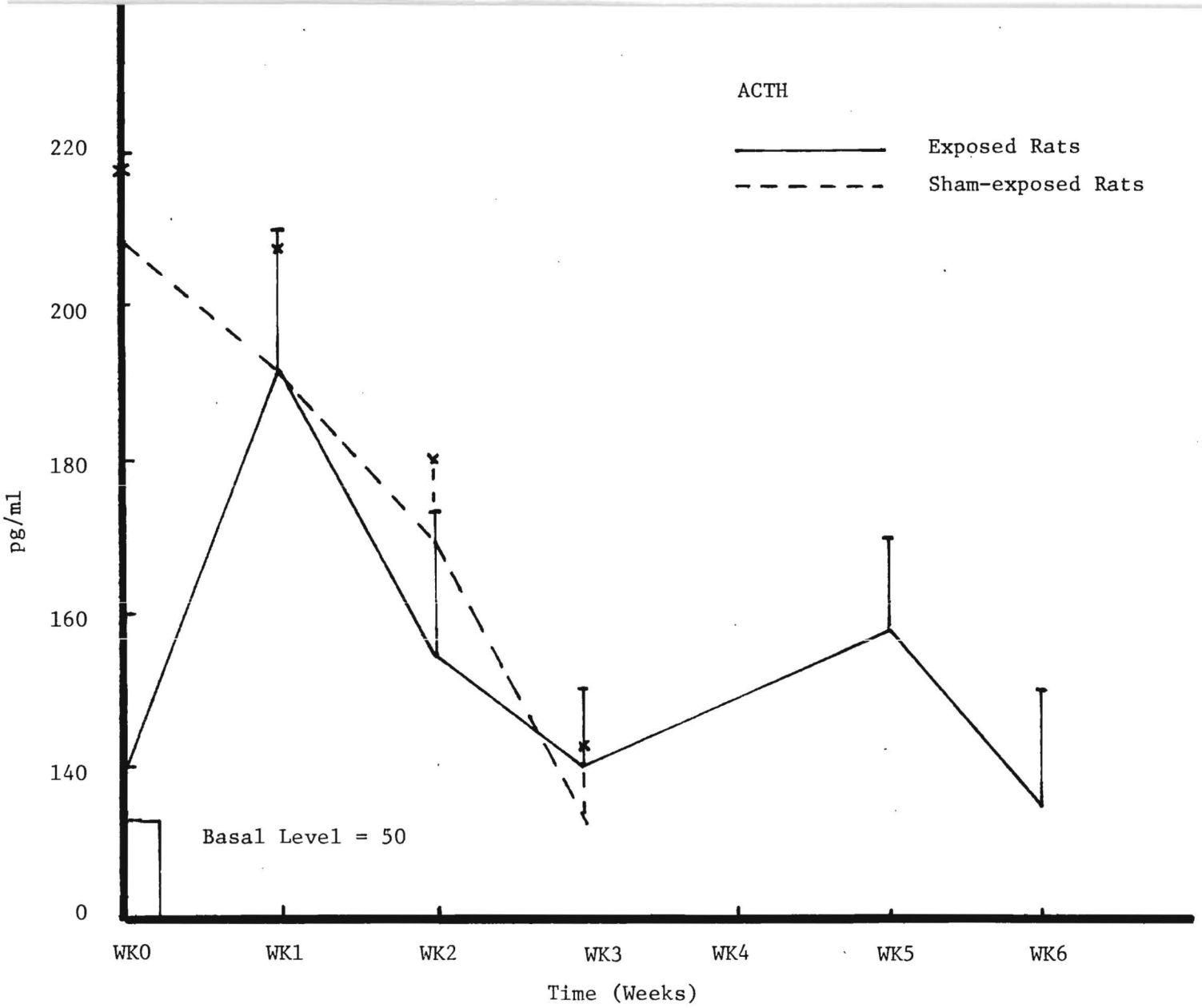


Figure 9. Variations in Plasma ACTH Levels.

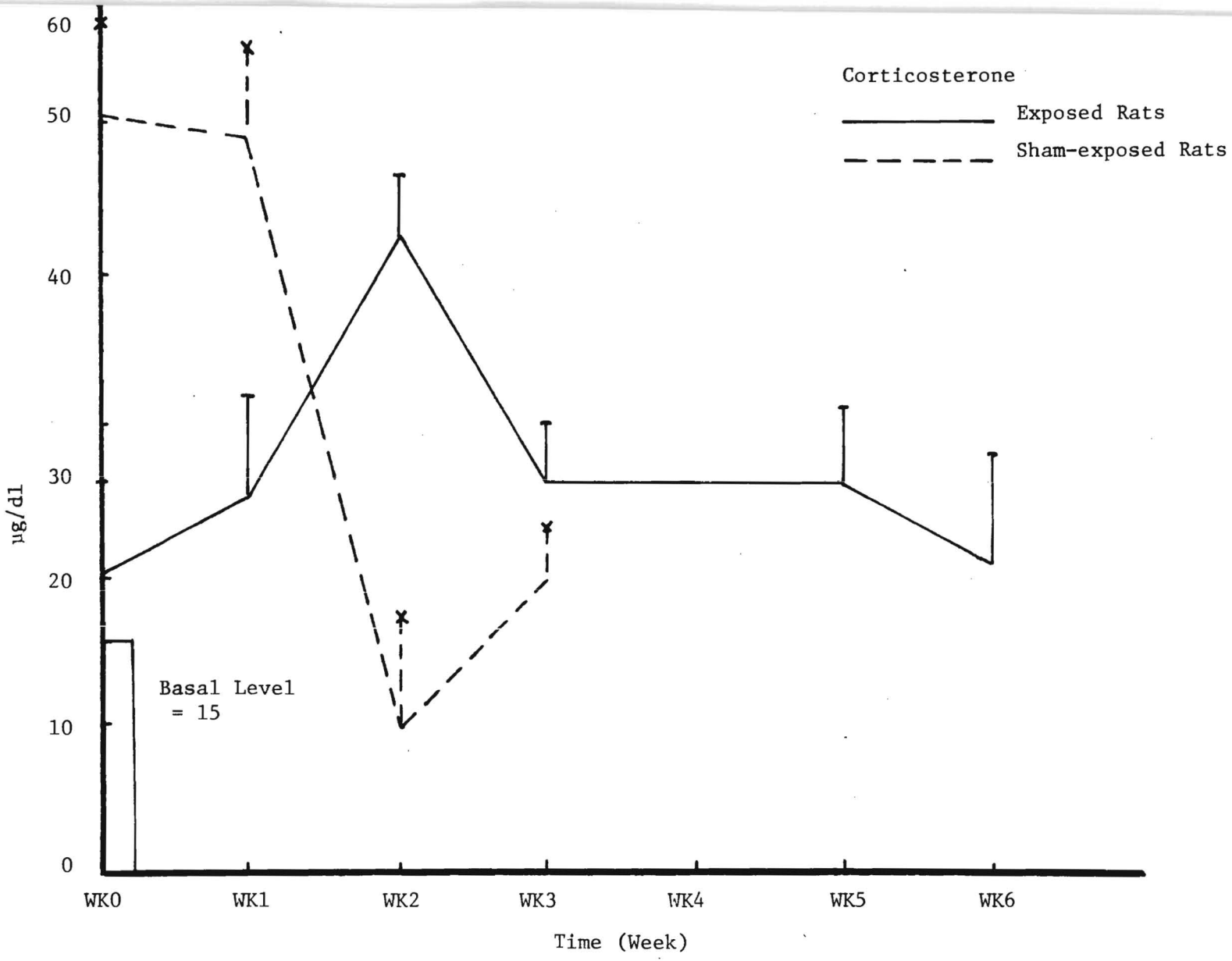


Figure 10. Variations in plasma corticosterone levels.

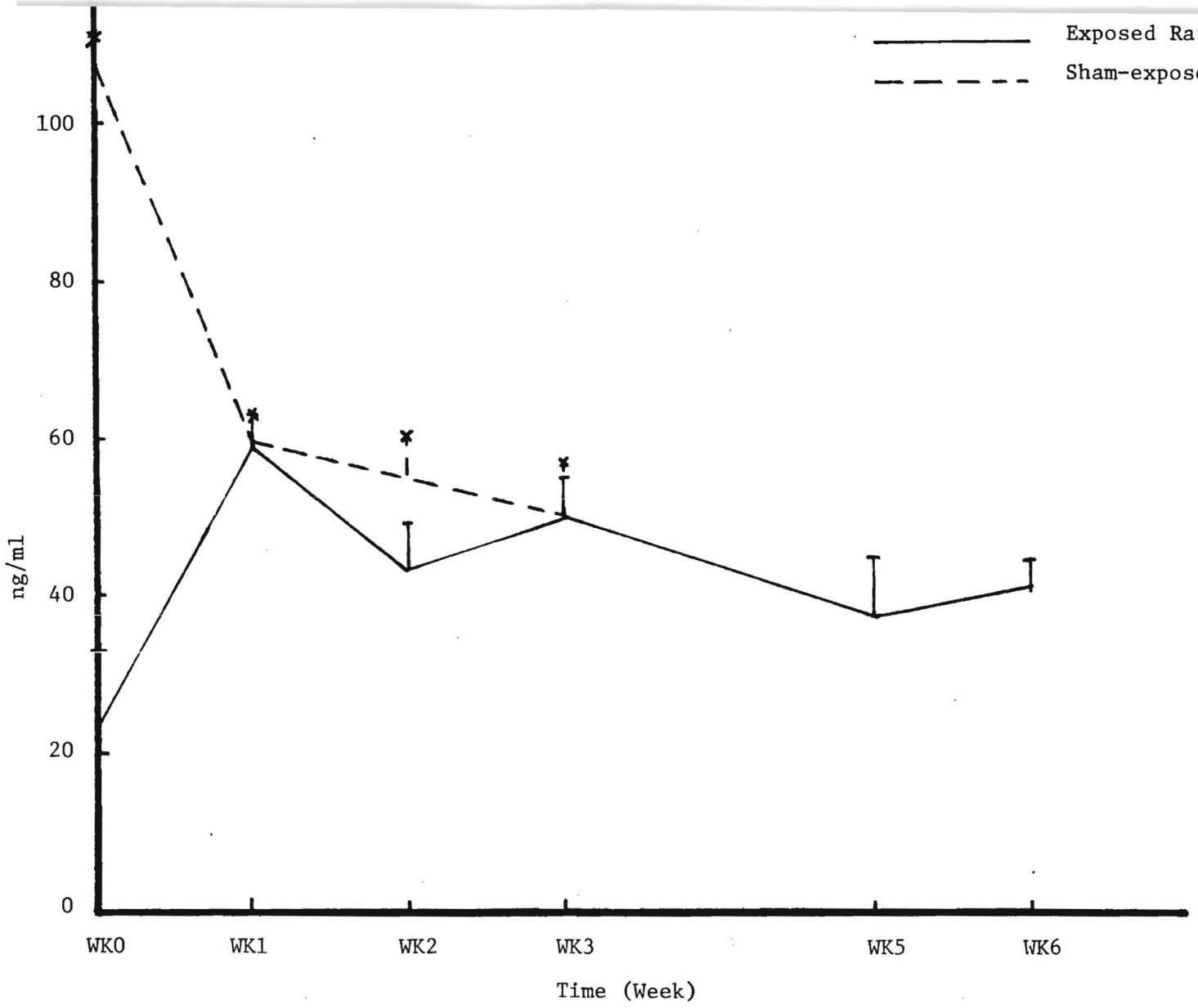


Figure 11. Variations in plasma prolactin levels.

and 11) represent the day the animals were placed for the third time in the Plexiglas boxes, blood samples were drawn for the first time, and RFR exposure was begun.

Results show an increased plasma level for all three hormones indicating an environmental stress possibly induced by RFR. The main peak for plasma ACTH occurred after one week of RFR exposure while the corticosterone peak occurred at the second week. While these two hormones returned to pre-exposure or near pre-exposure levels one week later, the plasma prolactin level remained somewhat elevated throughout the shakedown evaluation.

In summary, the results indicate that plasma ACTH, plasma corticosterone, and plasma prolactin were increased during early exposure (first and second week) to low-intensity RFR. The "stress hormones" then returned to their resting levels, suggesting adaptation to the new environment.

Results of all plasma hormone levels were available 10-to-14 days after the blood samples were drawn. This was found to be unacceptable and the flow of results between the Departments of Physiology and Pathology will be expedited to provide results within no more than four days.

Basal hormone levels as reported in the literature are also plotted in Figures 9, 10, and 11. These levels are an approximate average of a number of values presented as basal levels for the conditions under which, and techniques by which, they were measured.

During the first three weeks of the shakedown evaluation, arterial blood was drawn from an additional 25 cannulated rats and radioimmunuassays were performed. This blood was drawn on Monday of each week and two samples, rather than one, were drawn 20 minutes apart while the rats remained in the Plexiglas holding boxes. Plasma hormone levels were similar in both samples for each rats, thus demonstrating the adequacy and reproducibility of the radioimmunuassay techniques. Furthermore, the techniques of double blood withdrawal indicated that the main "stress" which elevated plasma hormones at the initial week was exposure to an unfamiliar holding box (lasting 20 minutes, or as in the case of second blood withdrawal 40 minutes) and to a lesser degree, removal from the cage. When the values of plasma hormones were decreased in Week 2 (third week of sampling), the difference between two samples was smaller, but again, the second sample did not show any decrease in values.

Weight data for the exposed and sham-exposed rats are presented in Figure 12. The sham-exposed rats entered the evaluation at a mean weight slightly less than the exposed rats. This difference was maintained throughout the evaluation without statistically significant variations as determined by the student t-test (p-values greater than 0.5 in each case). Comparisons of these data with weight data from other rats are not presented because no growth curves exist for cannulated rats housed in anything approaching the specialized Plexiglas cages used during this program.

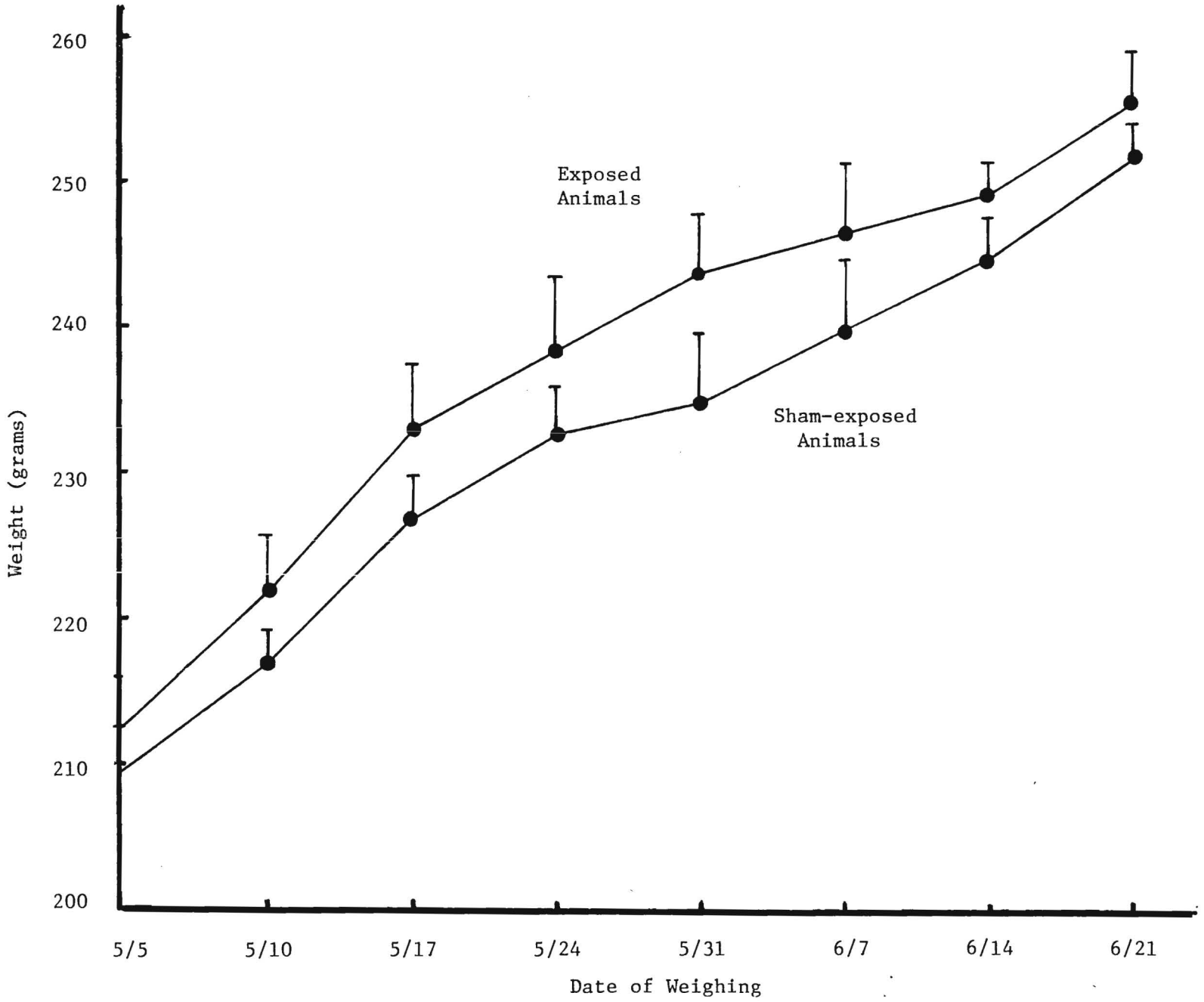


Figure 12. Weight for exposed and sham-exposed animals during the shakedown evaluation.

SECTION VIII
CRITIQUE

The primary purpose of this program was to conduct a six-week operation (shakedown evaluation) of the 435-MHz RFR Facility to assure that all tasks associated with a comprehensive long-term bioeffects study involving a large rat population could be adequately accomplished. In the proposal for the first long-term bioeffects study (Proposal No. EC-BR-1216, "Long-Term, Low-Level Bioeffects Study Using a Large Rat Population Exposed to 435-MHz RFR", submitted to AFSAM/RZP, 25 May 1982), a number of procedures based on results of the shakdown evaluation were incorporated. Now several additional modifications can be proposed as a result of new data collected after the proposal was submitted. These modifications are presented as a critique of procedures used during this program and recommended changes to these procedures.

- The specially-designed Plexiglas cages used to house rats in the Radiation and Control Rooms apparently used too few glass rods to form the floor. These rods were spaced 0.5 inches apart and possibly provided inadequate support for the rats. This resulted in a longer habituation period than would have otherwise been necessary and plasma hormone values that were above BMR levels. The number of rods in the floor has been doubled in experimental cages now being used to analyze the support provided by the cages and its influence on habituation and plasma hormone levels. It is expected that this analysis will indicate a need for improved flooring in cages used during follow-up studies.
- Persons involved in handling the rats and maintaining the Facility during this shakedown evaluation were not professionals working full-time on the program. Follow-on research programs will include a full-time person professionally trained as an animal caretaker. This will substantially improve the ability to handle large numbers of rats without inducing undue excitement. All animals will be handled by this person only, and the handling will involve use of a heavy glove placed underneath and around the animal's body.
- Both RFR-exposed and sham-exposed animals will be handled (placed in the Plexiglas holding box for 20 minutes) at least four times before initiation of the radiation.

- Microsamples of blood will be drawn from all RFR-exposed and sham-exposed animals three times before initiation of the radiation. Only after hormonal levels have fully stabilized will the actual study begin.
- Microsamples of blood will be drawn twice (day 2 and day 5) during Week 1 and Week 2 after initiation of radiation. Assay results from these blood samples will better define changes in plasma hormones during a time of possible acclimation.
- Cannulae will be sealed with a removable plastic plug (not heat sealed) that permits much longer use of the original cannulae and requires no extension parts.
- All animals will be completely undistrubed for 18 hours prior to blood sampling.
- As explained in Section VII.C, Experimental Results, the NIH kit for radioimmunoassay of rat growth hormone was not available for use during this program. The necessary kits have now been obtained with highly purified porcine growth hormone as the ^{125} I labeled antigen. Radioiodination of growth hormone will be carried out according to the methods established by D. Heber, W. Odell, H. Schedewie, and A. Wolfsen, Clin. Chem., 24:796,1978. It is noted that, for rat growth hormone assay, reagents are not commerically available. Reagents routinely used in clinical laboratories for human growth hormone determination by radioimmunoassay are not useable because of cross reactivity of rat growth hormone with corresponding human hormones.
- Active efforts are underway to implement a liquid chromatographic assay for plasma catecholamines. In the past, a radiometric technique routinely used in the clinical laboratory for assaying catecholamines in human plasma was experimental with. The technique involved several chromatographic and other steps that made it both tedious and time consuming. Further, it required 0.5 ml of plasma and it was not possible to scale it down. High performance liquid chromatography with electrochemical detection (Oka, et al., Clin. Chem., 28:646-649, 1982) offers several advantages over previous techniques (fluorometry, bioassay, calorimetry, and radioassay). This method is available for urine and brain

tissue catecholamines and will be adopted for plasma. There are indications that it can be scaled down to require only 50 µl samples.

- All assays will be performed under strict quality control rules. The same guidelines (controls, split samples, blind samples, etc.) employed in the clinical laboratory of Emory University Hospital will be applied. Participation in evaluation programs such as those sponsored by the Center for Diseased Control, the Association of Clinical Chemists, the College of American Pathologists, etc. will continue.

SECTION IX

REFERENCES

1. J. Toler, et al., "Feasibility Study to Determine Design and Construction Criteria for a 420-450 MHz Chronic RFR Exposure Facility for Rats," Georgia Tech Final Report on Project A-2228, Subcontract No. SCEEE-ARB-78-3, July 1979.

2. J. Toler, et al., "Prototype Circular Parallel Plate Facility for Chronically Exposing Large Rodent Populations to 420-450 MHz Radiofrequency Radiation, Georgia Tech Final Report on Project A-2392, Subcontract No. SCEEE-ARB-79-20, January 1980.

3. J. Toler, et al., "Construction of a 435-MHz Radiofrequency Radiation Facility for Long-Term Bioeffects Studies Involving Large Rodent Population", Georgia Tech Final Report on Project A-2650, Subcontract No. SCEEE-ARB-80-34, July 1981.

4. Antennas, J. D. Kraus, McGraw-Hill Book Company, New York, NY, 1950.

5. G. Sinclair, "The Patterns of a Slotted-Cylinder Antenna", Proceedings of the IRE, December 1948, pp. 1487-1492.

6. H. Bassen, et al., "A Miniature Broadband Electric Field Probe", Ann. NY Acad. Sci., 247:481-486, 1975.

7. V. Popovic, et al., "Technique of Permanent Cannulation of the Right Ventricle in Rats and in Ground Squirrels", Proc. Soc. Exp. Biol. Med., 113:559-602, 1963.

8. V. Popovic and P. Popovic, "Permanent Cannulation of Aorta and Vena Cava in Rats and Ground Squirrels", J. Appl. Physio., 15:727-728, 1960.

9. V. Popovic, et al., "Technique for the Introduction of Neutropenia and Granulocytosis in Rats", Exp. Hematology, 4:285-288, 1976.

10. W. B. Cannon and D. dela Paz, "Emotional Stimulation of Adrenal Secretion", Am. J. Physio., 27:64-70, 1911.

11. H. Selye, "Studies in Adaptation", Endo., 21:169-188, 1937.

12. A. W. Guy, et al., "Study of Effects of Long-Term, Long-Level RF Exposure on Rats: A Plan," Proc. IEEE, 68:92-98, 1980.

13. Z. R. Glaser and C. H. Dodge, "Review of Radiofrequency and Microwave Radiation Effects Research and Issues: 1977-1981", Abs. of BEMS 3rd Ann. Mtg., p. 31, 1981.

14. M. O. Carruba, et al., "Blood Sampling by Chronic Cannulation Technique for Reliable Measurement of Catecholamines and Other Hormones in Plasma of Consious Rats", J. Pharmacol. Methods, 5:293-303, 1981.

15. H. U. Buhler, et al., "Plasma Adrenaline, Naradrenline, and Dopamine in Man and Different Animal Species", J. Physiol. (London), 276: 311-320, 1978.

16. F. Depocas and W. A. Behrens, "Effects of Handling, Decapitation, Anesthesia, and Surgery on Plasma Noradrenaline Levels in White Rat," Cana. J. Physiol. Pharmacol., 55:212-219, 1977.

17. C. C. Chiueh and K. J. Kopin, "Hypersensitivity of Spontaneously Hypertensive Rat to Indirect Measurement of Blood Pressure", Am. J. Physiol., 234:H690-H695, 1978.

18. C. A. Blake, "Effects of Intravenous Infusion of TRH on Plasma TSH and Prolactin Concentrations in Rats", Proc. Soc. Exp. Biol. Med., 154:558-561, 1977.

19. S. W. Smith and R. R. Gala, "Influence of Restraint on Plasma Prolactin and Corticosterone in Female Rats", J. Endocr., 74:303-314, 1977.

20. G. Pinardi, et al., "Contribution of Adrenal Medulla, Spleen, and Lymph to the Plasma Levels of Dopamine-Hydroxylase and Catecholamines Induced by Hemorhagic Hypotension in Dogs," J. Pharmacol. Exp. Ther., 209:174-174, 1979.

21. B. B. Fredholm, et al., "Plasma Catecholamines, Cyclic AMP and Metabolic Substrates in Hemorrhagic Shock in the Rat", Acta Physiol. Scand., 105: 481-495, 1979.

22. S. B. Friedman, et al., "Plasma Corticosterone Response to Parameters of Electric Shock Stimulation in the Rat", Psycholsom. Med., 29:323-328, 1967.

23. M. L. Simon and R. George, "Diurnal Variations in Plasma Corticosterone and Growth Hormone as Related to Regional Variations in Norepinephrine, Dopamine, and Serotonin Content of Rat Brain", Neuroendoc., 17:125-132, 1975.

24. C. Gonzalez and T. Jolin, "Plasma and Pituitary Concentration of Growth Hormone in Male and Female Rats During a 24-Hour Period", Hor. Res., 14:130-137, 1981.

25. Y. Takahashi, et al., "Regulation of Immunoreactive Growth Hormone Secretion in Male Rats", Endoc., 88:909-917, 1971.

26. J. Seggie and G. Brown, "Coping with Stress: Parallelism Between the Effects of Septal Lesions on Growth Hormone and Corticosterone Levels", Biol. Psychi., 11, 1976.

27. C. Turpen, et al., "Stress-Induced Gonadotropin and Prolactin Secretory Patterns", Neuroendoc., 20:339-351, 1976.

28. W. P. Smotherman, et al., "Pituctary-Adrenal Responsiveness of Rat Mothers to Noxious Stimuli and Stimuli Produced by Pups", Ciba Foundation Symp., 45:5-25, 1976.

29. E. Zimmerman and V. Critchlow, "Effects of Diurnal Variation in Plasma Corticosterone Levels on Adrenocortical Response to Stress", Proc. Soc. Exp. Biol. Med., 125:658-664, 1967.

30. R. Ader and S. Friedman, "Plasma Corticosterone Response to Environment Stimulation: Effects of Duration of Stimulation and the 24-Hour Adrenocortical Rhythm", Neuroendoc., 3:378-386, 1968.

31. J. Seggie, et al., "Adrenal Stress Responses Following Septal Lesions in the Rat", Neuroendoc., 16:225, 1974b.

32. S. Eden, et al., "Plasma Levels of Growth Hormone in Female Rats of Different Ages", Acta Endoc., 88:676-690, 1978.

33. K. Takahashi, "Effects of Various Traumatic Stresses on Growth Hormone Release in Pentobarbital Anesthetized Rats", Neuroendoc., 26:1-7, 1978.

34. J. Urquhart, "Physiological Actions of Adrenocorticotrophic Hormone", in Handbook of Physiology. Endocrinology, IV, Part 2, edited by R. O. Grepp, et al., Washington, D.C., Am. Physiol. Soc. 1974, Chapter 27.

35. J. Mattheij and T. van Pijkeren, "Plasma Prolactin in Undistrubed Cannulated Male Rats: Effects of Perphenazine, Frequent Sampling, Stress, and Casturation Plus Oestrone Treatment," Acta Endoc., 84:51-61, 1977.

36. B. N. Natelson, et al., "Humoral Indices of Stress in Rats", Physiol. Behav., 26:1049-1054, 1981.

37. R. C. Kvetnansy, et al., "Effect of Handling and Forced Immobilization on Rat Plasma Levels of Epinephrine, Norepinephrine, and Dopamine- -Hydroxylas", Endoc., 103:1868-1874, 1978.

38. M. Hennessy and S. Levine, "Sensitive Pituitary-Adrenal Responsiveness to Varying Intensities of Psychological Stimulation", Physiol., Behav., 21:295-297, 1978.

39. W. F. Ganong, "The Central Nervous System and the Synthesis and Release of Adrenocorticotropic Hormone", in Advances in Neuroendoc., Univ. of IL Press, Urbana, IL, pp. 92-149, 1963.

40. H. Wakabayashi, et al., "Effect of Pentobarbital and Ether Stress on Serum Prolactin Levels", Proc. Soc. Exp. Biol. Med., 137:1181-1193, 1971.

41. C. L. Sun, et al., "Comparison of the Effects of 2-Deoxyglucose and Immobilization of Plasma Levels of Catecholamines and Corticosterone in Awake Rats", Endoc., 105(1):306-311, 1979.

42. R. Kvetnansky and L. Mikulaj, "Adrenal and Ruinary Catecholamines in Rats During Adaptation to Repeated Immobilization Stress", Endoc., 87:738-743, 1970.

FINAL TECHNICAL REPORT

GT/EES PROJECT A-3055

OPERATIONAL EVALUATION OF A NEW 435 MHZ RADIOFREQUENCY RADIATION FACILITY

By

J. Toler and V. Popovic

Submitted to

AIR FORCE SCHOOL OF AEROSPACE MEDICINE
Code SAM/RZP
Brooks Air Force Base, TX 78235

Under

Contract No. F33615-81-K-0620

August 1982

GEORGIA INSTITUTE OF TECHNOLOGY

TABLE OF CONTENTS

LIST OF FIGURES

SECTION I

INTRODUCTION

Over the last decade there has been a significant amount of research concerned with whether biological effects, either hazardous or otherwise, are induced in living systems as a result of exposure to radiofrequency radiation. A large portion of this research has involved either engineers or biologists exposing small animal populations to specific pulsed- or continuous-wave radiation environments for relatively short time periods while monitoring biological endpoints presumed to be sensitive to the radiation insult. Although these research efforts have been helpful, there is a very real sense in which they have raised more questions than they have answered. The reasons for this situation are numerous and include the following:

- In many instances, the persons conducting the research have been qualified engineers or biologists, but not both; consequently, key aspects of one discipline or the other were not adequately considered.
- The animal populations have often been too small to yield statistically significant data.
- Exposure facilities have been limited to the point that it has been difficult to study the same biological endpoints during exposure to radiation with the same propagation mode but different electromagnetic parameters.
- No rationale has been provided to explain why the selected endpoints should be responsive to radiofrequency radiation. Also, the procedures used in accessing these endpoints have been capable of masking any radiation-induced bioeffects.
- Dosimetry determinations have not been adequate to accurately characterize the absorbed dose of radiation.
- The exposure frequency and animal species were such that a meaningful extrapolation of the data to the man-model was not possible.

To overcome difficulties such as these, the Air Force School of Aerospace Medicine (AFSAM), Radiation Sciences Division, is sponsoring bioeffects research programs involving interdisciplinary teams exposing large animal populations to real-world radiation environments in facilities versatile enough to generate both pulsed- and continuous-wave fields. The exposure

frequency and animal species are selected to assure extrapolation of the resulting data to the man-model. The endpoints monitored are stress-sensitive and therefore expected to be responsive to effects induced by environmental insults such as radiofrequency radiation.

The AFSAM-sponsored program in the Biomedical Research Division at Georgia Tech's Engineering Experiment Station has involved four individual studies. In the first of these studies [1], several potentially satisfactory facility concepts were analyzed theoretically, and the circular, parallel-plate concept in a multi-tier configuration was identified as the most technically and economically feasible. The second program [2] involved construction of a prototype, single-tier set of circular, parallel plates and development of a slotted cylinder antenna to feed the plates. The plates and antenna were then evaluated by measuring radiation patterns in an anechoic chamber as a function of plate separation, frequency, radial position from the plate center, vertical position between the plates, slot parameters, etc. The third program [3] provided a full-scale, 435-MHz circular, parallel plate radiofrequency radiation facility. The facility consists of two, four-tier stacks of circular, parallel plates located in adjoining, absorber-lined rooms. Additionally, an assay room, transmitter room, utility room, storage room, computer room, and office area, all colocated with the rooms housing the circular, parallel plates, is provided. Each tier of circular, parallel plates accommodates 25 rodents; therefore, one four-tier configuration can be used to expose 100 rodents to a 435-MHz environment while the other configuration can house 100 control rodents. This program also provided Plexiglas cages for the rodents and a 435-MHz transmitter with pulse- and continuous-wave outputs of 5 KW and 200 W, respectively. The fourth program is described in this report and had, as its primary objective, the performance of a "shakedown" evaluation to familarize project personnel with operation of the exposure facility and analysis of the blood samples. This program was undertaken by an interdisciplinary team of engineers from Georgia Tech and medical professionals from Emory University. In addition to performing the shakedown evaluation, this team also integrated several remaining subsystems into the facility and trained biomedical technologists in both cannulation techniques and microassay procedures. Detailed efforts undertaken during this program are described in the following sections of this report.

SECTION II

SUMMARY DESCRIPTION OF FACILITY

Prior to detailed descriptions of efforts related to the shakedown evaluation, it was considered beneficial to provide a summary description of the Radiofrequency Radiation (RFR) Facility. This description will be particularly helpful later in the report when procedures for handling the experimental animals are described. As a key part of the Facility, characteristics of the exposure field in the Radiation Room are also described.

A. Facility Description

The RFR Facility consists of eight rooms located in the basement of the Baker Building on the Georgia Tech main campus. The floor plan and layout for these rooms was specifically designed to provide an essentially self-contained facility for bioeffects studies involving large rodent populations and long-term RFR exposures. The Baker Building is a three-story, brick building in which a large variety of electronic research programs are conducted; however, this program is the only one involving experimental animals. Heating and air conditioning systems for the RFR Facility are isolated from those in the remainder of the Baker Building, and exhaust air is routed outside the building through a large plenum. Power for lights in the rooms that house exposed and sham-exposed animals is routed through a timer that can be programmed to provide any desired lighting cycle. Hot and cold water supplies are taken from primary sources and are therefore unaffected by service outages that might cause loss of water in other Baker Building locations. Rooms housing exposed and sham-exposed animals are constructed in a manner that provides electromagnetic shielding to assure that stray radiation does not reach other research areas. To accomplish this, walls in the Control and Radiation Rooms utilize aluminum-backed sheet rock in their construction. This aluminum backing is joined together with conductive tape along all seams between adjacent panels of sheet rock; therefore, an electrically-conductive aluminum shield is provided for the walls. For the ceilings, individual 2-foot by 2-foot acoustical tiles have been removed, covered with aluminum shielding, and remounted in their metal support frames. Since the facility floor is on ground level, it requires no shielding

The RFR Facility floor plan is shown in Figure 1. During the shakedown evaluation, the rooms designated A and B in Figure 1 housed exposed and sham-exposed rats, respectively. Identifical 12-foot diameter circular, parallel-plate structures were located in both rooms. These structures consisted of four-tier stacks of circular, aluminum plates with an 18-inch separation distance maintained betwen them by 1.5-inch diameter plastic rods [2]. These plates functioned as open-ended waveguides and were fed with slotted cylinder antennas located at the plate centers [3]. The walls of both rooms were also lined identically with pyramidal-shaped microwave absorbing material that provided a -30 dB reflectivity at 500 MHz. The timers were used to cycle the lighting on a 6:00 am - 6:00 pm schedule. Temperature in these rooms was maintained at 70 $\pm$ 2°C during the first four weeks of the evaluation, and then increased to 74 $\pm$ 2°C during the remaining weeks. Humidity was maintained at 48 $\pm$ 2 percent throughout the evaluation. Room C in Figure 1 was used as a storage and maintenance area. Items stored included rat chow, Styrofoam soil trays, custodial supplies, extra cages, etc. The work area in this room was used primarily for repairing damaged cages. The cage washer with its entry and exit tables was housed in Room D. Room E was the area to which the rats were transferred from their large Plexiglas exposure cages into small Plexiglas holding cages in preparation for drawing microsamples of blood. This room also housed the electronic balance and was therefore used when the rats were weighed. After transfer into the small Plexiglas cages, the rats were moved into Room F where a 15-minute or more acclimation period was provided before microsamples of blood were drawn. During this acclimation period, the door to Room F was closed and only the biological technician remained in the room. Room G provided a buffer area between the Control/Radiation Rooms and routine activity in the hallway outside the Facility. It also housed the Data Acquisition System into which weight data for each rat was entered, and desk space for the engineering technician. The 435-MHz transmitter plus spare parts and a work bench were located in Room H. Coaxial connectors on the transmitter top provided power output ports for the four antennas located in the Radiation Room (Room A). Cables connected to these four connectors were routed up through the ceiling of Room H and across to the slotted cylinder antennas in Room A.

The above summary description shows the RFR Facility to be a complex of eight contiguous rooms that provide space for

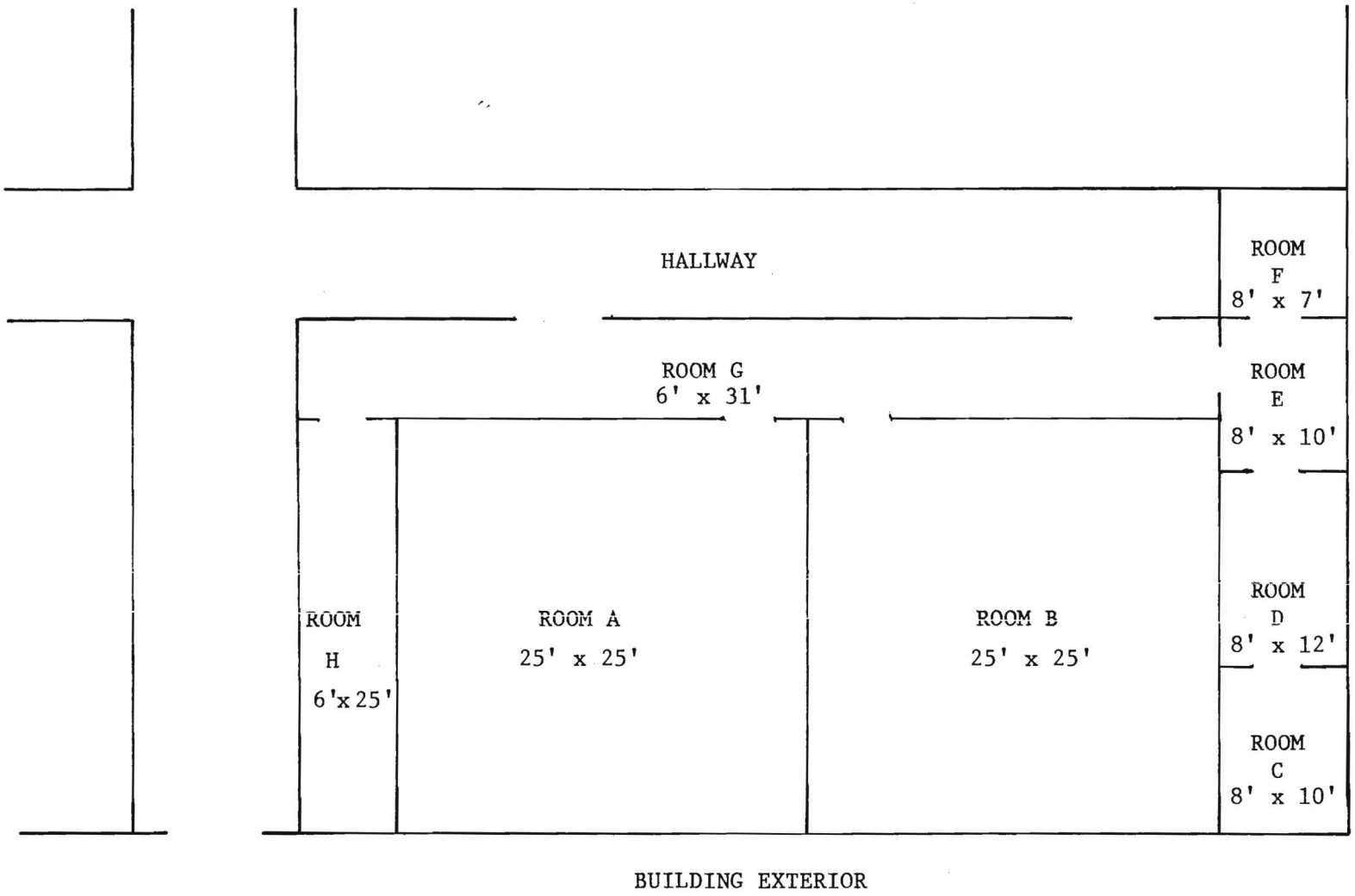


Figure 1. Floor Plan for the Radiofrequency Radiation Facility.

- housing large populations of exposed and sham-exposed rats in two identical and adjoining rooms,
- both storage of suppplies and spare parts, and for maintenance of equipment and cages,
- a commercial cage washer,
- transfer of rats from exposure cages into holding cages,
- weighing the rats,
- drawing microsamples of blood from the rats,
- a buffer zone between the Control/Radiation Rooms and routine activity in the Baker Building, and
- housing and operating the 435-MHz transmitter.

B. Exposure Field Description

Slotted-cylinder antennas were selected to feed the circular, parallel-plate waveguides because they generate a horizontally-polarized field with an essentially-constant amplitude pattern. Performance of these antennas can be intuitively understood by referring to Figure 2, where the antenna is first considered to be a slot in a flat metal sheet [4]. This slot is fed at its center with a coaxial cable and the current directions are indicated by arrows. The sheet metal may then be formed into a U-shape and finally into a cylinder, with the coaxial feed cable located inside the cylinder. If the impedance around the cylinder circumference is sufficiently low, current will flow in horizontal loops around the cylinder. Under these conditions, the slotted-cylinder functions as an antenna radiating a horizontally polarized field, the amplitude of which is dependent on the cylinder diameter. In general, the radiated field tends to be greater on the cylinder side where the slot is located; however, if the cylinder diameter is a sufficiently small part of a wavelength (approximately 0.1λ), the radiated field in the horizontal plane becomes essentially uniform. If the cylinder diameter is increased to the point of becoming a significant part of a wavelength, the field in the region of the shadow cast by the cylinder becomes small. Generally, as the cylinder diameter becomes large, the horizontal field approximates a cardioid [5].

On a previous program [2], the interactive relationship between slot width, slot length, cylinder diameter, and cylinder wall thickness was investi-

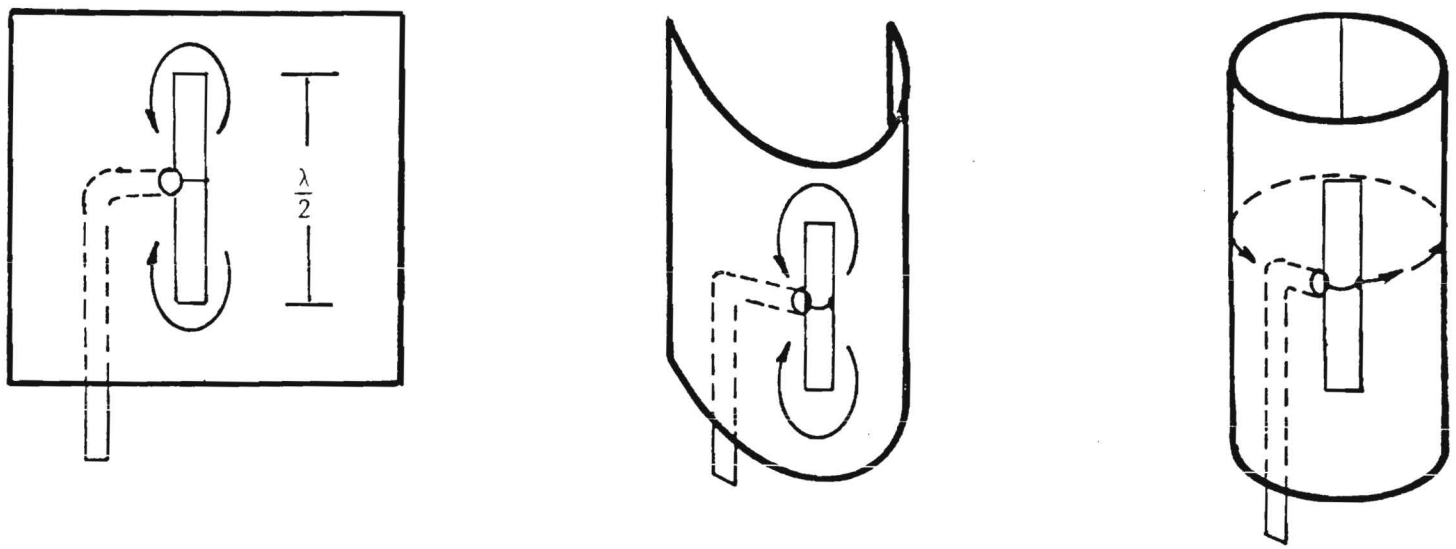


Figure 2. Formation of a slotted-cylinder antenna (see Reference 4).

gated, and it was concluded that a slotted-cylinder antenna with the following features would provide a suitable feed for the circular, parallel-plate waveguides:

- cylinder diameter: 4 inches
- cylinder wall thickness: 0.125 inches
- slot length: 14 inches, and
- slot width: 0.125 inches.

Four sets of circular, parallel-plate waveguides were stacked one above the other to provide a structure for exposing a large population of rats, and an identical structure was provided for housing control rats. The 12-foot diameter of the plates made it possible to position 25 Plexiglas cages around the inside periphery of each set of plates (see Figure 3) while maintaining a cage center-to-cage center spacing of 18 inches. This separation distance resulted in intercage scattering of the exposure field being undetectable with small dipole probe antennas and a sensitive receiver tuned to the exposure frequency. The spacing between individual sets of plates in each structure was 18 inches since, at the 435-MHz exposure frequency, this distance was greater than one-half wavelength, but less than one wavelength. This wavelength relationship assured that only the lowest-order TE-mode propagated outward in concentric circles about the slotted-cylinder feed antennas located at the plate centers. The electric field vector is necessarily zero at the plate surfaces, thereby preventing mutual coupling of fields between adjoining sets of plates. An extensive number of radiation pattern measurements were made with the resulting data confirming TE_{01} propagation in which the vertical component of the electric field vector was typically 17 dB below the horizontal component (see Figure 4) at 435 MHz.

During the shakedown evaluation, the transmitter was connected to slotted-cylinder antennas at levels 2 and 3 of the circular waveguides in the Radiation Room. The remaining two transmitter outputs were connected to dummy loads. The transmitter output was adjusted to provide a 1.0 mW/cm^2 exposure field at a position half way between the plates at levels 2 and 3. This exposure field was measured with a Narda Model Probe every 30 degrees around the plate circumference to determine uniformity of the exposure field. As in previous measurements, the power density varied by less than $\pm$ 1 dB around the plates at both levels. These variations were reconfirmed in measurements

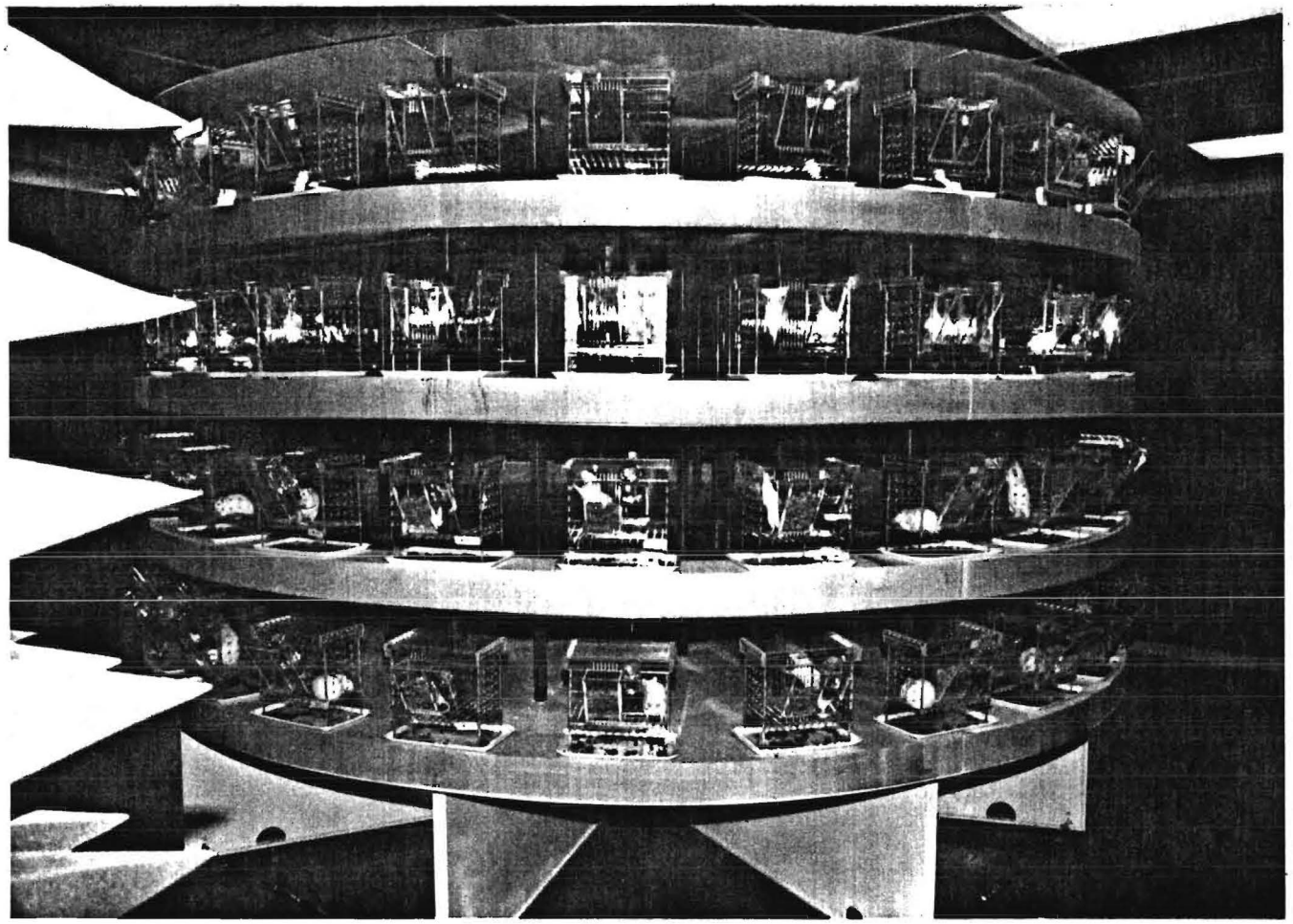


Figure 3. Rats Positioned on Circular, Parallel Plate Waveguides.

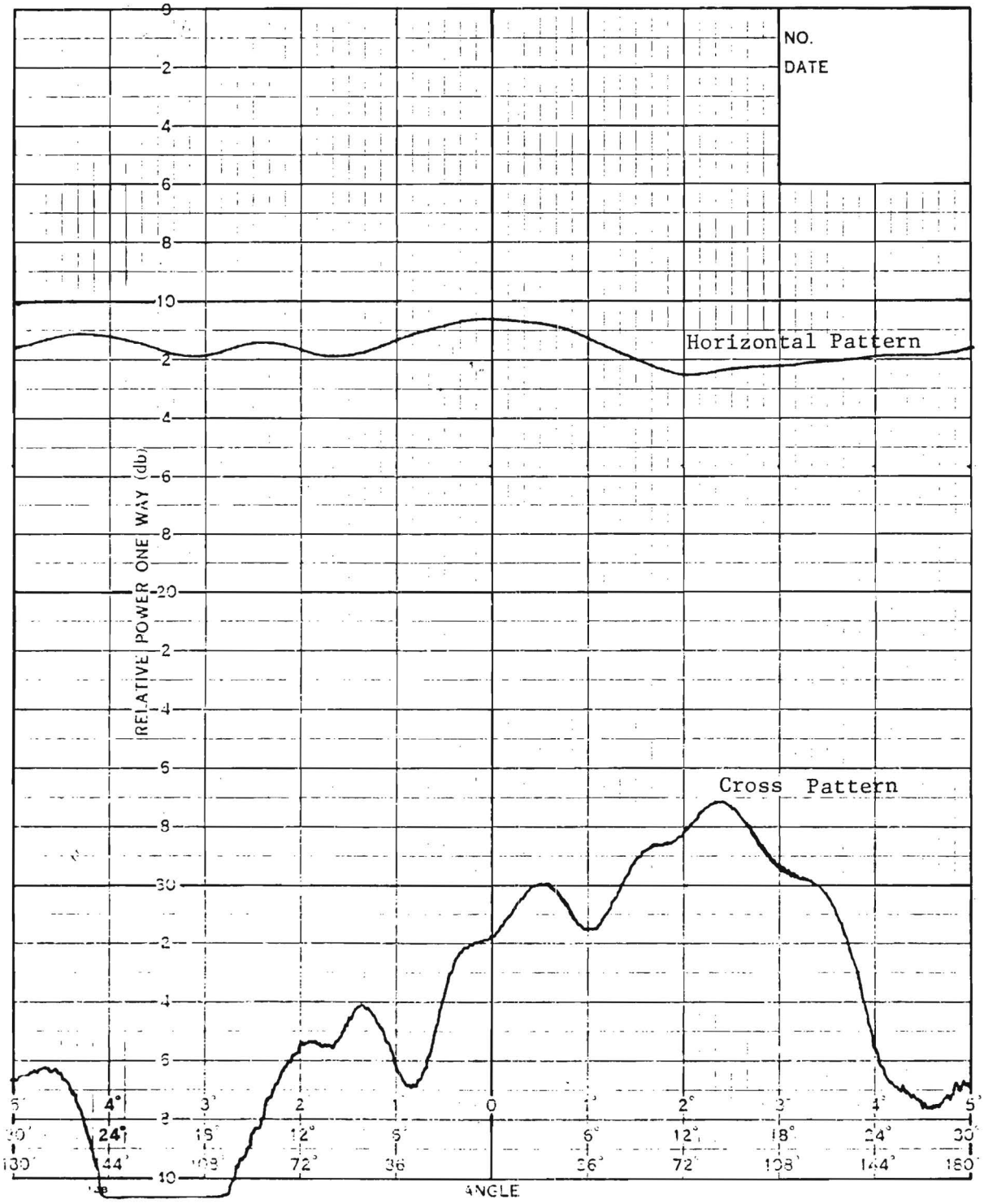


Figure 4. Typical radiation pattern at 440 MHz obtained with prototype, circular, parallel-plate waveguide and slotted-cylinder antenna.

made later with a three-dimensional I-beam electric field probe on loan from the Bureau of Radiological Health [6]. With this probe, the signal processing and display device was located outside the Radiation Room and interconnection with the probe was provided by a fiber optic cable.

When the space provided by eight contiguous rooms is considered, along with independent heating, air conditioning, lighting, and water supply systems plus open-ended waveguides for exposing/sham-exposing experimental animals, it is seen that the RFR Facility provides an essentially self-contained area for conducting a wide variety of either long-term, low-level, or short-term, high-level bioeffects studies.

SECTION III
DATA ACQUISITION SYSTEM

In developing overall performance capabilities for the data acquisition system, it was noted that the system must (1) automate the data logging procedure to the maximum extent possible, (2) monitor the status of the exposure fields, (3) provide a home alert in case the exposure fields drop below predetermined levels during non-work hours, (4) perform basic statistical analysis of the data, and (5) interface with the Georgia Tech large mainframe computers for more extensive data processing. In conversations with AFSAM personnel, it was also agreed that the system should be compatible with those used in chronic bioeffects studies at AFSAM and the University of Washington. Compatibility with the AFSAM and University of Washington systems dictated a system configured around the industry standard S-100 bus. This bus standard was originally known as the "Altair" bus appearing in the MITS Altair computers in 1975. Because of its tremendous design flexibility, it was quickly adopted by many microcomputer manufacturers and is now regarded as the most-used busing standard ever developed by the computer industry. Physically, it consists of a set of 100-contact edge connectors mounted to a common motherboard and wired in parallel. Modules plugging into the edge connectors are circuit boards measuring 5 x 10 inches.

With the above system requirements in mind, a review of commercially-available, S-100 compatible computers was conducted, with the results that the Northstar Horizon and Cromemco System Two units were determined acceptable. Both offered a system core consisting of S-100 motherboard, Z-80 8-bit processor chip as the Central Processing Unit (CPU), 64 K Random Access Memory (RAM), two drives for 5.25-inch, double-sided, dual-density disks, a controller, a cabinet, and necessary power supplies. Retail prices for the Northstar Horizon and Cromemco System Two units were $4,330 and $4,695, respectively; however, both systems were available at discounts of approximately $750 from several different computer marketing centers. As capabilities of the two systems were compared in detail, it was noted that either could be used for this program, but that the Cromemco System Two offered 390 kbytes per drive and a motherboard with 21 board slots. Comparable features of the

Northstar Horizon system were 380 kbytes per drive and 12 board slots. Based on these differences in technical specifications, the Cromemco System Two unit was purchased and the system configuration shown in Figures 5 and 6 was developed.

In describing the data acquisition system shown in Figure 5, it is first noted that the S-100 bus was originally designed for use with a CPU based on the 8080 microprocessor; however, in the Cromemco System Two, the CPU has been designed around the Z-80 microprocessor. Cromemco's designation for this Z-80 based CPU is ZPU. The ZPU in Figure 5 has the following technical specifications:

Processor: 4 MHz version of the Z-80 microprocessor

Clock Rate: 2 or 4 MHz (switch selectable)

Instruction Set: 158 instructions including the 78 instructions of the 8080 microprocessor

Power On Jump: Jumper-wire enabled

Power-On Jump Locations: 16 switch-selectable locations

Wait-State Generations: 0-4 jumper-selectable wait states

M1 Wait State: Jumper wire selectable

Bus Compatibility: S-100

Power Requirements: + 8 VDC at 1.1A

The S-100 bus interfaces the Z-80 CPU module to as many as 20 additional memory, input/output (I/O) or other processor modules. Signals associated with this bus can be grouped into four categories as follows: (1) power supply, (2) address, (3) data, and (4) clock and control. The power supply signals involve three unregulated DC voltages as follows: +8, +18, and -18 volts. Since the main power supplies are unregulated, power supply regulation must be provided on individual circuit cards. For the address signals, there are 16 address lines that allow direct addressing of 65,536 words of memory space. Tri-state TTL drivers are used to drive the address bus. To handle data signals, the S-100 bus has two directional data buses, each eight bits wide. All clock and control signals are standard TTL levels and there are three clock signals on the S-100 bus.

The 64 kbyte RAM is provided by a S-100 bus-compatible read/write memory board with the following specifications:

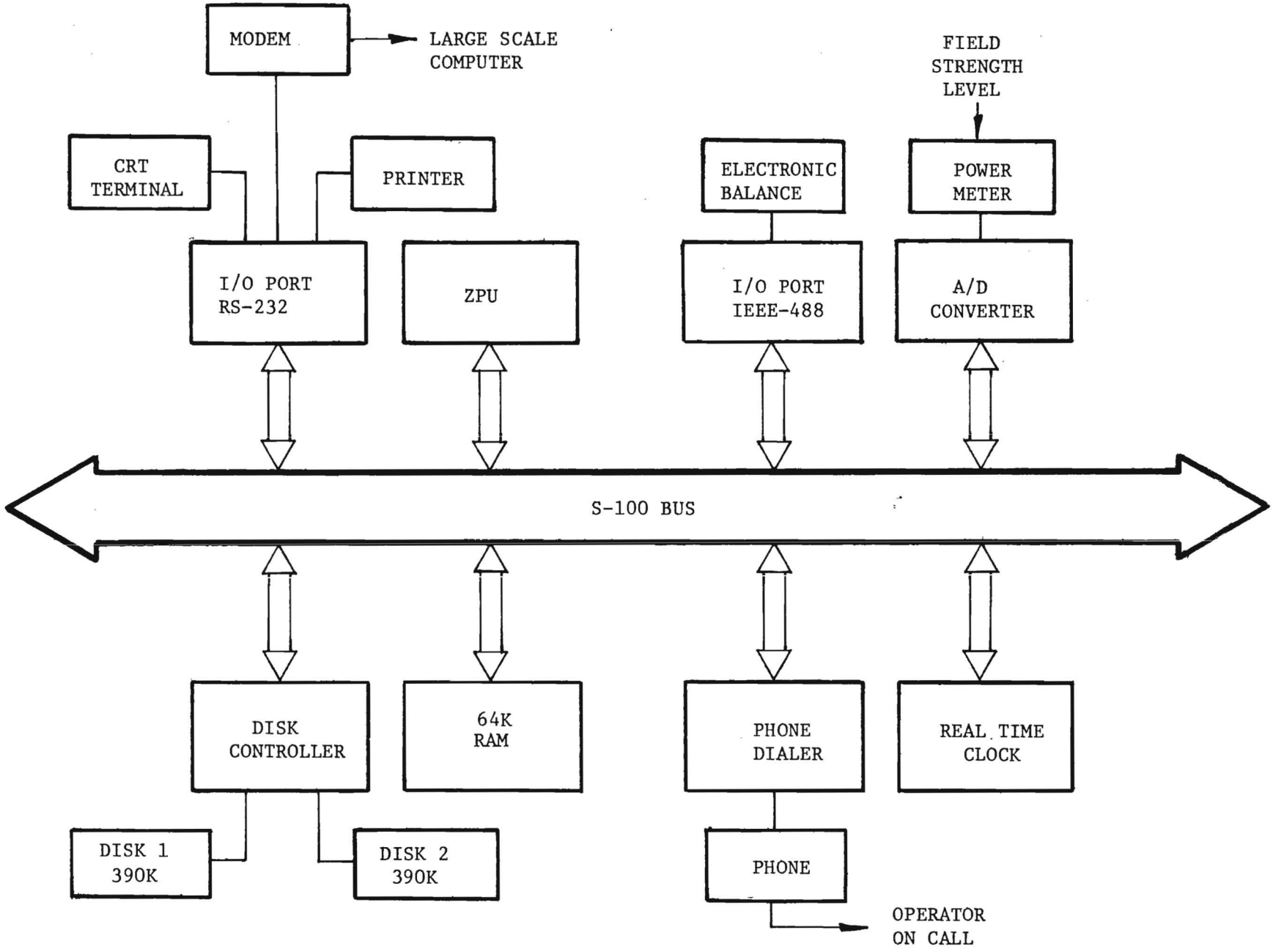


Figure 5. System Configuration for Cromemco System Two Data Acquisition System.

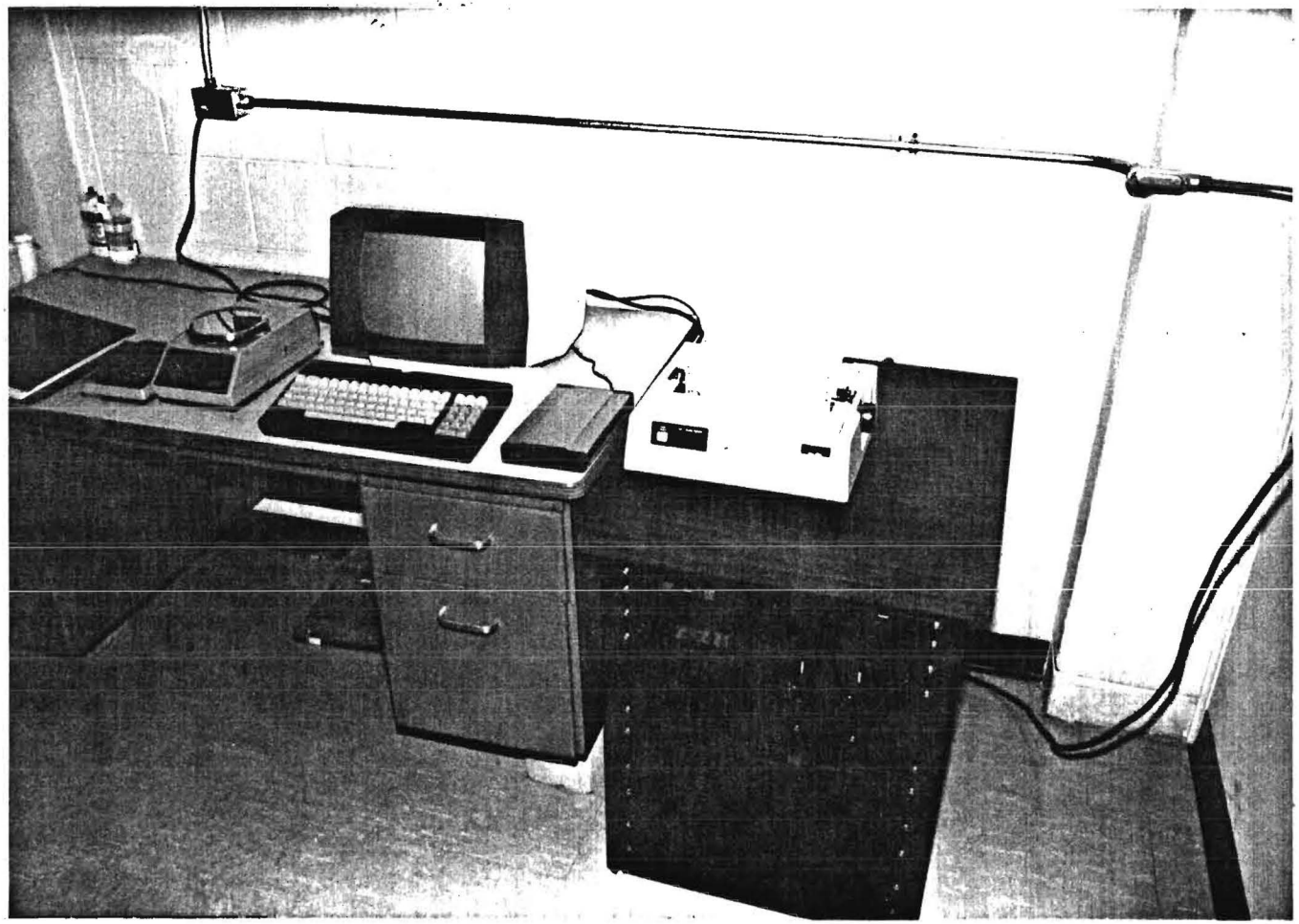


Figure 6. Photograph of Data Acquisition System.

Memory Capacity: 65,536 Bytes

Memory Type: TMS 1416-15, 16K X 1 dynamic RAM

Memory Access Time: 250 nanoseconds (max)

Wait States at 2 MHz: None required

Wait States at 4 MHz: None required

Bus Compatibility: S-100

Power Requirements: +8, +18, and -18 volts

Interfacing of the CRT terminal, modem (for communicating with Georgia Tech mainframe computers), and printer with the S-100 bus is provided by a Cromemco D+7A Input/Output Module. Specifications for this module are as follows:

Analog Input Ports

- Number: 7
- Input Voltage Range: -2.56 to +2.54
- Resolution: 8 bits
- Conversion Time: 5.5 microseconds

Analog Output Ports

- Number: 7
- Output Voltage Range: -2.56 to +2.54
- Resolution: 8 bits
- Conversion Time: 5.5 microseconds

Parallel I/O Port

- Input Port: 8 bits
- Output Port: 8 bits
- Input Load: one TTL equivalent
- Output Drive: 10 TTL loads

Bus Compatibility: S-100

Power Requirements: +8, +18, -18 volts

A Pickles and Trout Model P&T-488 interface permits the electronic balance to communicate with the S-100 bus. This interface appears as four I/O ports that are addressed as consecutive ports with the first port address an integer multiple of 4 (0,4,8,...). These ports allow the ZPU to manipulate the data, handshake, and bus management lines of the IEEE-488 bus.

The clock-calendar card shown in Figure 5 is a Scitronics, Inc. Model RTC-100 unit and is used to provide the timing signals necessary for monitoring field strength in the Radiation Room on an interrupt-driven basis. This

monitoring function also requires an A/D converter to digitize the output of the power meter and communicate it to the S-100 bus.

In order to provide a hard copy of the data, the data acquisition system includes a Microline Model 82A Printer. This unit employs an impact dot matrix print system in which characters are a 9 x 7 matrix of dots. The printing direction is bidirectional and the printing speed is 120 characters per second with the normal character spacings of 10 and 16.5 characters per inch. The unit prints alphanumeric characters and symbols plus lower-case English letters and symbols. A tractor unit is provided to feed paper into the printer. Interfacing of the printer with the S-100 bus is via a low-speed serial port based on the RS-232 code.

SECTION IV

CAGE WASHER SYSTEM

After reviewing the technical specifications, cost, size, etc., of cage washers from several manufacturers, the Southern Cross Model 900-A Dyna Jet Washer was purchased and installed in Room D of the RFR Facility as shown in Figure 7. This washer is of stainless steel construction and provides fully automatic wash, rinse, and final rinse cycles for all types of animal cages and accessories. A tempered safety glass viewing window is installed in the washer front. Loading of the washer is accomplished from the left (soil) side through a guillotine, pass-through door with a safety interlock feature that prevents washer operation until the door is closed. Exit is on the right (clean) side through a pass-through door identical to the one on the entry side. Cage washing is provided by water jets from motor-driven, rotating, stainless steel manifolds located above and below the wash compartment. A 35-gallon detergent tank is located below the wash compartment and used for the wash cycle. This tank contains a heavy-duty heating element with an external control so wash water temperature can be varied from room temperature up to 190^{o}F. The rinse cycle uses hot tap water from the building utility supply. For final rinse, a 27-gallon tank with an automatic water level controller is provided. External plumbing provides for the introduction of special final rinse fluids (distilled water, dionized water, etc.) and disinfectants.

Automatic reset timers control the wash and rinse time intervals as follows:

Wash Cycle: 0 to 10 minutes

Rinse Cycle: 0 to 10 minutes

Final Rinse Cycle: 0 to 1 minute.

These timers are wired such that any cycle can be omitted by adjusting the cycle timer to the "off" position. Once the timers have been set to the desired cycle durations, the start button is pushed and the wash operation is automatically continued through all cycles.

To facilitate wash and rinse operations, special-purpose stainless steel racks for the Plexiglas cages and glass water bottles are provided.

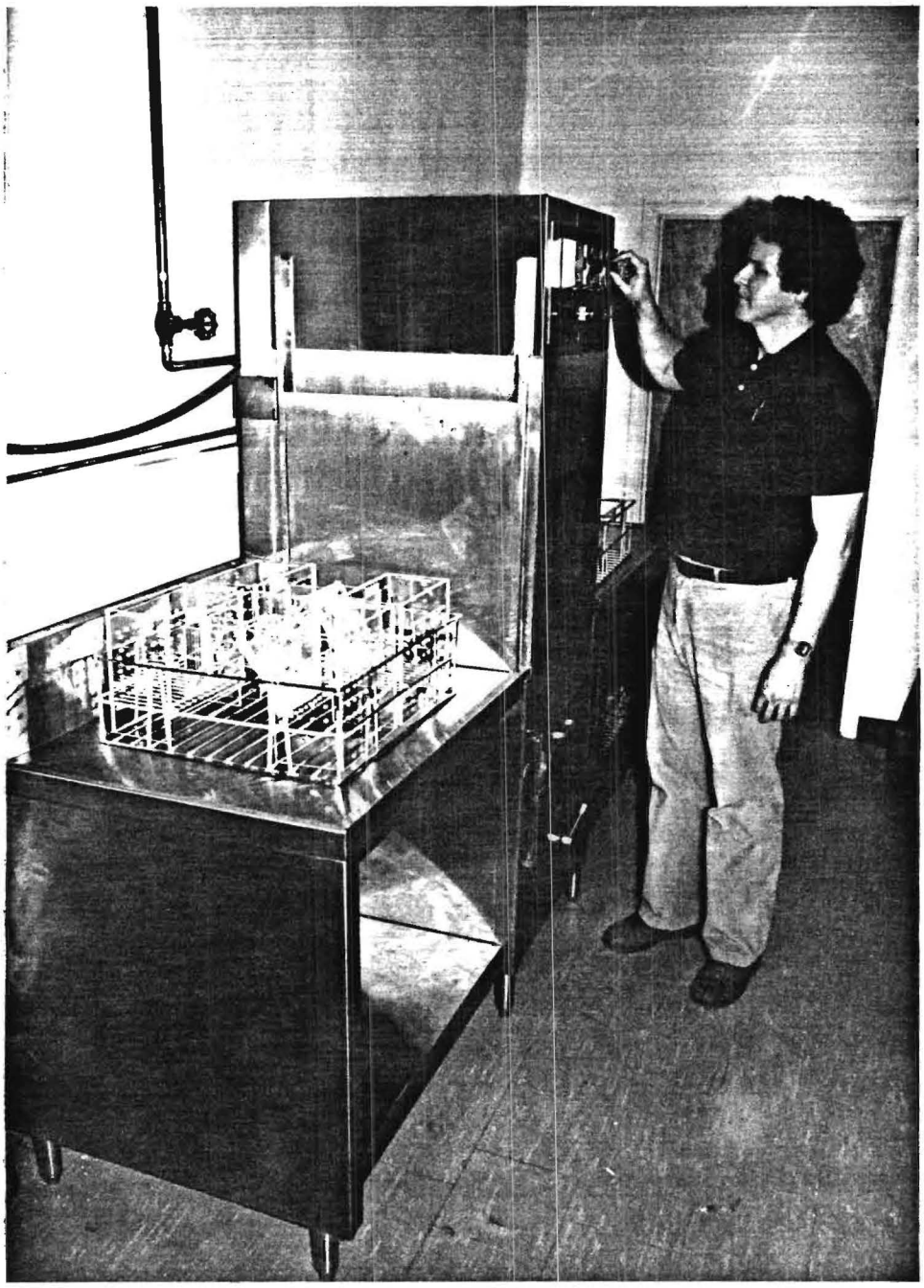


Figure 7. Photograph of Cage Washer.

These racks hold four cages and 49 bottles, respectively, in the wash compartment of the washer. The bottle rack provides a cover of stainless steel mesh to prevent water jets in the washer bottom from forcing bottles out of the rack during wash and rinse cycles.

SECTION V

ELECTRONIC BALANCE SYSTEM

Another important system purchased and installed during this program was the electronic balance. After reviewing technical capabilities of balances from several manufacturers, the Sartorius Model 1203 MP Balance with built-in microprocessor, variable integration time, and locked-in readout was purcahsed. This balance offered a weighing range and readibility of 0 to 4000 grams and 0.1 gram, respectively. In addition to the balance, a Sartorius Model 704201 Keyboard Programmer and Model 705306 Cable box were also purchased. The resulting electronic balance system was used to accurately weigh excited rats and automatically store the weight data in the data acquisition system. The keyboard programmer made it possible to program the balance for a variety of different weighing procedures including net weight, mean weight, average weight, accumulated weight, and difference from a predetermined average weight. The weight displayed by the balance was actually the average of a programmed number of individual weighings performed over either a fast (0.5 seconds), normal (1 second), or slow (2 seconds) integration time. Once the programmed number of weighings was completed, the average weight was locked in and displayed without fluctation on a large, seven-segment digital readout. The taring capability of the balance was instantaneous and was accomplished by touching the tare switch on the balance front. When the weight data displayed on the readout had achieved stability, a small "g" symbol appeared next to the weight value and indicated accurate data.

In most instances during the shakedown evaluation, the balance system was not interfaced with the data acquisition system because the wrong interface module was ordered. Late in the shakedown evaluation, the correct interface module was received and the following program was used to accurately weigh the rats:

KEY	FUNCTION
g	Tare the balance Calls up stored regular weighing program
	Select weighing range on balance (0-400 grams)

10	Key-in the desired number of individual weighing (in this case, 10)
CS	Store the desired number of individual weighings
25	Key-in rat identification number (in this case, number 25)
#	Transfer the number 25 to data output
	Place rat on balance pan
A	Start program. The balance automatically weighs Rat # 25 ten times, displays the number of individual weighing, stores and adds up the individual weight values, calculates the mean weight, displays the mean weight, and transfers the mean weight value to the data output.
	Tare the balance
	Place next rat on the balance pan
A	Start program

SECTION VI

EXPERIMENTAL ANIMALS

All exposed and sham-exposed animals were carriers of chronic aortic cannulae. The distal end of the cannula was positioned in the aortic arch (only 1-2 mm) while the proximal end was exteriorized at the back of the neck. Implantation of the cannula was done under nembutal anesthesia. Once implanted, the cannulae remain patent for months, some for the rat's lifespan [7-8]. Over 16,000 rats have now been cannulated in Dr. Popovic's laboratory. Adverse effects of cannulation of the aorta have never been described, nor has published criticism of the procedure been offered.

During this program, 150 Spraque-Dawley rats were used. The rats were delivered from the breeding colony (Harlan Sparque-Dawley, Madison, WI) to Dr. Popovic's animal quarters at Emory University. The animal quarters are fully isolated, being private quarters to Dr. Popovic's laboratory. A rigid schedule of visual observation, care, maintenance, and cleanliness is enforced, resulting in ultraclean animal quarters and research laboratories. The lighting cycle in these quarters was 8 am to 8 pm and the temperature was maintained at 24 $\pm$ 1^{o}C. In these animal quarters, the rats were provided food (Purina Rat Chow) and water ad libitum and carefully screened for health problems while growing to a body weight of 170 to 180 g. While growing to this weight, health status was monitored daily, and body weight was measured twice weekly. Rats that did not follow normal body weight curves (established for this strain during years in Dr. Popovic's laboratory) were eliminated as were those with an increased total white blood cell count or an unusual differential count (0.3 ml of arterial blood sampled once per week).

When the rats reached a body weight of approximately 175 g, 75 of them were randomly selected for cannulation (all animals were cannulated by the same person, biomedical technologist C. Honeycutt, MSc). The cannulated rats were returned for seven days to their individual cages in the animal quarters for recovery from the cannulation. Fifty cannulated rats, in addition 50 others that were not cannulated, were randomly selected and then transferred to the RFR Facility at Georgia Tech where daily visual observations continued and the rats were weighed twice weekly. In the RFR Facility, the rats were

housed in Plexiglas cages specially designed for this program and positioned on the circular, parallel-plate waveguides in the Control and Radiation Rooms, where their acclimation to the new environment took place. A laboratory record was opened for each rat, and both environmental factors and health activity were placed in the animal's record. Neither the biomedical technologist who drew blood samples nor the assay personnel who analyzed the blood samples were able to distinguish between exposed and sham-exposed rats. Baseline data were obtained from the blood samples drawn and assayed for the biologoical endpoints proposed earlier.

SECTION VII

SHAKEDOWN EVALUATION

The shakedown evaluation was conducted to assure that the numerous tasks associated with (1) cannulating and managing a large population of rats, (2) operating the exposure facility, (3) drawing microsamples of blood, and (4) assaying the blood samples could be satisfactorily accomplished. The evaluation was conducted over an eight-week period and is described in the following paragraphs in terms of the biological endpoints monitored, the experimental procedures used, and the results obtained.

A. Biological Endpoints

The effects of long-term exposure to low-level RFR on rats and other mammals was discussed in Georgia Tech Proposal No. EC-BR-1216, "Long-Term, Low-Level Bioeffectts Study Using 200 Cannulated Rats Exposed to 453-MHz Radiofrequency Radiation," pages 7-11. This discussion concluded that, with a few exceptions in which artifacts were suspected, long-term exposure to low-level RFR does not appreciably alter biological parameters. This, of course, does not necessarily mean that all of the parameters stay unchanged; it is possible that some more sensitive or more reactive endpoints do change. It is also possible that, after changing (which indicates a specific physiological distrubance), the endpoints revert back to normal or near-normal levels because of adaption on the part of the biological system. An example of endpoints likely to exhibit this behavior is hormones. The rationale for monitoring hormones is that exposure to RFR represents an environmental condition that might be stressful to biological systems. Certain humoral changes are known to be sensitive responders to stress. Of course, to detect the early beginning of such humoral change would be of interest because of the crucial role of hormones in providing physiologic control of all vital processes and because it would provide an early clinical test to detect any possible harmful effect of RFR. The inter-relationship among hormones provides both the flexibility and small gradations in response that are fundamental to homeostatic control. Hormones play a crucial integrative and regulatory role in major processes including energy production, control of body fluids, growth, development, and others. Control of hormone secretion

is especially important in maintaining homeostasis. Such control is achieved by a negative feedback mechanism in which the hormone producing a biological effect achieves a sufficient magnitude to inhibit its further secretion. This negative feedback system consists of multiple components involving detection of a real or threatened homeostatic imbalance, a means of signaling secretion from secretory cells, responder organs or organ systems, and shutoff of secretion when adequate hormone levels are reached. By these mechanisms, the delicate physiologic balance required for homeostasis is maintained.

For this study, adenohypophyseal adrenocorticotropic hormone (ACTH), adrenal corticosteroid cortisosterone, catecholamines epinephrine and norepinephrine, growth hormone and prolactin were chosen as biological endpoints. Selection of these hormones was based on their common link to the existence of exogeneous stressors. This common link is evident when literature dealing with possible effects of environmental stress on plasma levels of these hormones, and possible mechanisms that might be involved, is reviewed. Furthermore, some of the results obtained in stressed animals, and the importance of determining basal hormone levels for correct evaluation of the imposed stress, are also evident. In the following paragraphs, some of the more significant research findings in this area are summarized.

It is generally accepted that catecholamines, ACTH, corticosterone, growth hormone and prolactin are good indicators of stress. This is true for man and it is true for many animals, especially for rats - a species studied more than any other. The concept of stress has been widely accepted as a specific somatic response to damage, or threat of damage, by a wide variety of environmental agents. This concept was first suggested by the observation made in 1911 by Cannon and de la Paz. They showed that the adrenal medulla releases hormones in the cat during the emotional excitment associated with exposure to a barking dog [10]. In 1936, Hans Selye demonstrated evidence of a second endocrine system, the pituitary-adrenal-cortical axis [11]. This system, responding often to a more subtle psychic and physical stress, was able to induce a more global and profound influence on metabolic functions.

Other endocrine systems (in addition to those involving the adrenals) can respond to stimuli also. In light of recent work, it seems that no endocrine system is entirely free from the influences of stress. Despite

these recent advances implicating virtually all endocrine systems, altered pituitary and adrenal function in relationship to the handling of stress has remained the central focus for present research.

In this respect, rats have been studied more often than any other animal species, as already mentioned. The literature showed that mostly Sprague-Dawley rats were used, with Wistar rats used in a few cases. In most investigations, the rats were sacrificed by decapitation before and after stress, plasma was collected, and then assayed on the hormonal level. In a very few investigations, retro-orbital venous plexus punctures were used [12]. Blood sampling after cutting the tail was used more often [13]. In a somewhat larger number of investigations, the rats were bearers of venous catheters (PE 50, 51, 60, 61, 62) and were used a few hours or, at the most, one or two days after cannulation [10, 40]. Carotid artery cannulae were used more often [14-19] than venous cannulae, but also only a few hours (at most, a day or so) after cannulation. It is believed that sampling blood from semi-chronic cannulae does not affect basal level of hormones; however, there are no studies to show when animals have fully recovered from anesthesia and surgery. In one study [15], the level of catecholamines was found to be the same in arterial and in venous blood. Repeated collection of 0.5-ml blood samples three times in 30 minutes equaling 1.5 ml, or nine times equaling 4.5 ml in 12 hours, did not change the values of basal catecholamines [14], a finding difficult to accept. It has been shown that a decreased blood volume increases catecholamine levels in plasma [20,21]. Some researchers used fresh, and some frozen, blood [22,23]. The plasma levels of "stress hormones" showed a clear circadian (approximately 24 hours) rhythm [11,12]. Circadian increases of plasma corticosterone [24,25] were large, with perhaps a six-fold difference observed between the lowest (early morning) and highest (late afternoon or early evening) values [26]. The plasma hormonal levels were increased after stress [27-31], but plasma growth hormone level in the rat was decreased after stress [26,32,33]. Some hormones, for instance ACTH, increased much after stress, sometimes ten-fold, but other hormones increased only slightly as, for instance, corticosterone [34]. Plasma prolactin was already increased two minutes after a mild stress [35]. It is generally accepted [36] that some hormones (for instance, catecholamines) respond to increasing magnitudes of stress in a step-wise fashion (i.e., respond

monotomically), while others (for instance, corticosterone and prolactin) respond in an all-or-none fashion [27], but not all investigators agree with the second part of this statement. Some investigators felt that plasma corticosterone increased with increasing levels of stress in a step-wise fashion [37], just as catecholamines do. Hennessy and Levine were able to prove that "corticoid levels can sensitively reflect differences in the intensity of stimulation" [38]. The response of the pituitary-adrenal axis to stress [27-31] was striking. After short stress, the increased plasma level of hormones had a duration of about 30 minutes [39] or less, depending on the strength and duration of stress. Some investigations suggested that rats which had not been subjected to daily handling showed a more dramatic stress-increased serum prolactin level [27], and that training (periodic handling) lowered the level of "stress hormones" [14], but there are other reports that handling (for four days in male rats) might increase prolactin response to stress [40]. For corticosterone, the biggest increase resulted from merely placing the rat in the test chamber [22]. Blood sampling in one rat affected the level of plasma "stress hormones" of other rats in the same room. Immobilization increased plasma catecholamines and corticosterone in conscious rats [17,37,41-46]. Stress increased plasma ACTH [47] and lead to pituitary-adrenal activation [48]. Even after a two-hour immobilization, the plasma hormone values were still at the same high level as in the early beginning of the stress [49]. Repeated stress decreased reaction of "stress hormones" [50] causing "habituation". Values of plasma catecholamines and plasma prolactin were higher after decapitation than when blood was sampled from catheters [45,47]. Plasma growth hormone level, like adrenal steroids, has been found to be very responsive to stress [51-53], demonstrating a rapid fall after the stress. It seems likely that growth hormone has circadian rhythmicity [51] which is inversely correlated with diurnal levels of corticosternone [23] and other homrones. However, not all investigators agree with the existance of circadian rhythm in growth hormone release [25,54] because of the inherent large variability of the data. Growth hormone (and other stress hormones) is released in an episodic fashion in resting (nonstressed) rats. Thus, mean growth hormone levels exhibited characteristically high variability [55]. Besides the studied hormones, plasma renin activity [56] and gonadotrophins might also be useful indicators of induced stress. Of

course, catecholamines are important controllers of renin release [57] and thus, catecholamine determination without renin activity measurements might be sufficient.

B. Experimental Procedures

During this program, experimental procedures were developed for monitoring hormone levels in a large population of rats exposed to pulsed-wave RFR at a frequency of 435 MHz. The hormone levels were monitored by assaying microsamples of blood drawn from the rats via their cannulae. The studied hormones respond to an exogenous stress in a manner similar to the dose-response relationship, i.e., the larger the stress, the greater the change in the plasma of stress hormones. Further, to study changes in ACTH, corticosterone, and prolactin, it is sufficient to draw only 0.3 ml of arterial blood from the chronic aortic cannula. There is no wasting of blood, a very small amount of blood is drawn weekly, samples consist of adequately mixed blood, the same rat can be used as its own control, and the rats can be followed before, during, and after RFR exposure lasting weeks or months.

On Monday, 26 April 1982, the rats were received at the Georgia Tech RFR Facility and began a two-week acclimation period. During this time, the temperature and humidity in the Radiation and Control Rooms were continuously monitored and recorded using calibrated meters provided by the Physical Plant. A typical chart from one of the recorders is presented in Figure 8 and shows that temperature and humidity were maintained at $72 \pm 2^{\circ}F$ and 50 ± 2 percent, respectively. During this two-week period, rats from the exposure group were handled (placed in the small Plexiglas boxes) numerous times as part of their habituation process.

Two weeks after receipt at Georgia Tech, the rats were considered to have completed their acclimation period and the shakedown evaluation with RFR began. Microsamples of blood were drawn from the cannulated rats on this day, and on each of the following five Mondays, between the hours of 10 am and 2 pm. During these hours, the plasma levels of the measured hormones are at their lowest values. The experimental procedure involved de-energizing the 435-MHz pulsed-wave (pulse rate = 1000 pps, pulse rate = 1.0 microsecond, exposure power density = 1 mW/cm^2) transmitter while a member of the program staff (animal caretaker) entered the Radiation Room with a cart designed to accommodate eight Plexiglas cages. (It is noted that this staff member

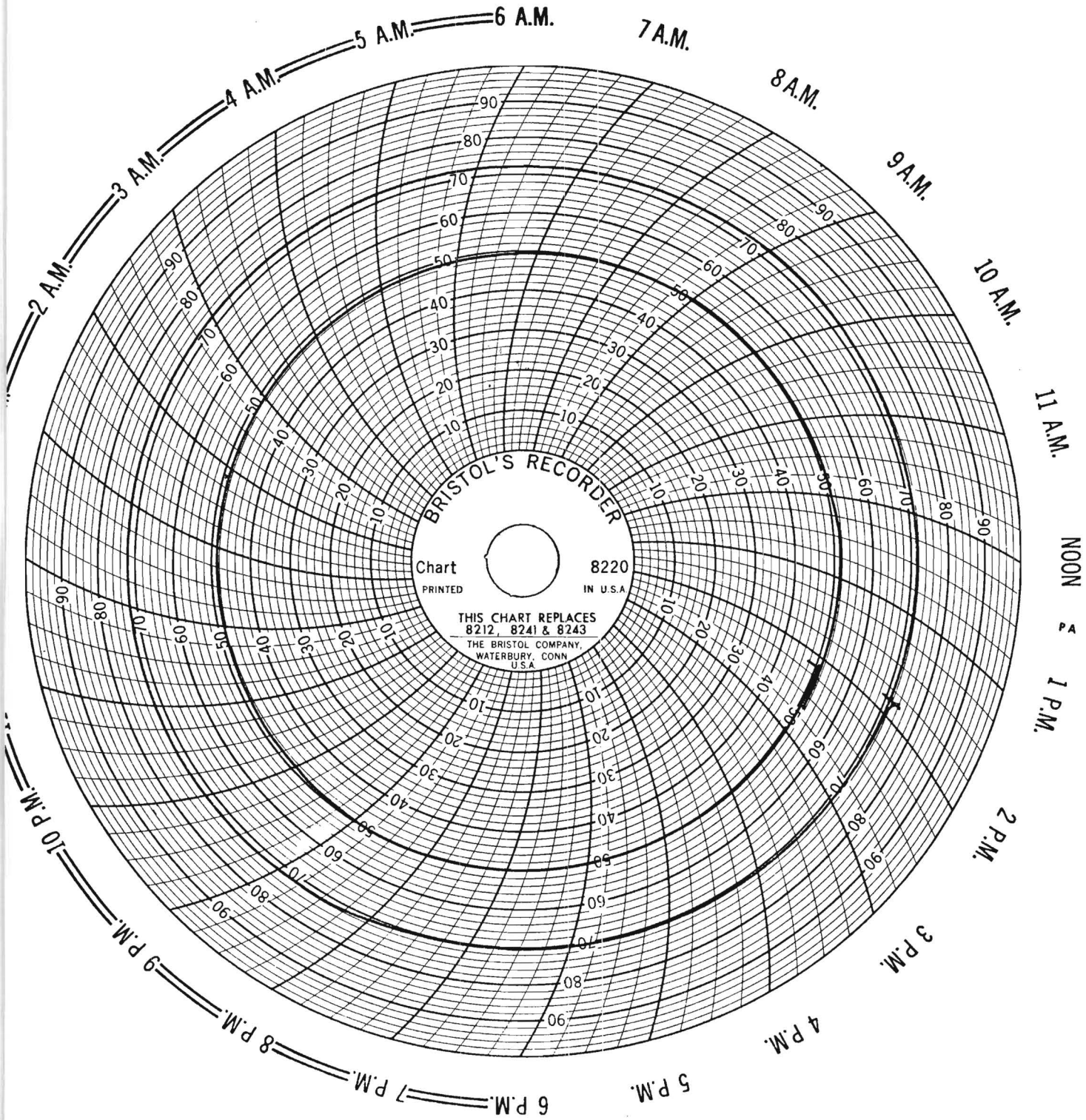


Figure 8. Chart Showing Temperature and Humidity in the RFR Facility.

was the only person authorized to work with the rats during procedures that involved the drawing of blood samples). Eight numbered cages were removed from the circular, parallel-plate waveguides, placed on the cart, and transferred to Room E, Figure 1. Once the staff member and rats were out of the Radiation Room, the transmitter was energized again. In Room E, the eight rats were transferred to small, black, Plexiglas boxes just large enough to house an individual rat and provide a comfortable space large enough that the rat can turn around but doesn't struggle to escape. The Plexiglas tops for these boxes were slotted so the cannulae could extend exterior to the box and be accessible for blood sampling. These small Plexiglas boxes were pre-numbered to correspond to the numbers on the larger Plexiglas cages used to house the rats while in the Radiation Room. Placement of the rats in the small Plexiglas boxes was done with slow, deliberate movements and in a manner that caused a minimum excitement of the rats. When all eight rats had been transferred to the small Plexiglas boxes, they were then moved to Room F, where a settling down period of at least 15 minutes began. Once the settling down period of approximately 15 minutes had passed, the rats appeared to rest comfortably (sleep, in most cases) and blood samples could be drawn during this resting period. Studies conducted during earlier programs have shown that this resting period extends for approximately four hours before the rats become restless. During this period, (and the following period during which blood samples were drawn), only the biomedical technologist was permitted in Room F. Also, the door to the room was closed to prevent sounds from other areas within the Facility from exciting the resting rats. After 15 or more minutes, a 0.3-ml blood sample was drawn with the cannula protruding through the slot in the box top. Mosquito hemostats padded with PE 240 plastic tubing were then used to clamp the cannula slightly below its heat-sealed tip. These hemostats were used to open and close the cannula, as desired, during the remainder of the procedure. The heat-sealed tip was snipped off and the animal's blood pressure slowly forced the heparinized saline solution and blood from the cannula. The heparinized saline solution (0.5 cc heparin--1000 units/ml from beef lung -- per 30 ml of saline solution) was used to fill the cannulae at the time of their implantation. When the heparinized saline solution cleared the cannula tip, a 30 guage needle attached to a 1 cc tuberculin syringe was used to draw 0.3 ml of undiluted blood.

The drawn blood was then transferred to Sarstedt capillary collection tubes calibrated for a 0.3 ml volume and treated with EDTAK to prevent clotting. These tubes were placed in a collection rack submerged in crushed ice where they were maintained until transfer to the assay laboratory. A different syringe and needle were then used to refill the cannula with heparinized saline solution and heat was applied to seal the tip.

A new needle and syringe were used to draw blood from each rat. Also, a new needle was used to refill the cannula with heparinized saline solution after each blood drawing.

This procedure was repeated for each of the eight rats. The biomedical technologist then transferred the rats back to Room E. While blood was being drawn from the first eight rats, a second group of eight rats was removed from the Radiation Room, transferred to small, black Plexiglas boxes, and were ready to begin their settling down period in Room F. As blood was drawn from this second group of rats, the first group was weighed using the electronic balance in Room E. The weight data were entered in the data acquisition system described in Section V. After being weighed, the first group of rats was placed in clean Plexiglas cages and carefully returned to their marked positions on the waveguides in the Radiation Room, and a third group of eight rats was transferred to Room E, in preparation for having their blood samples drawn.

By repeating the above procedure, blood samples were drawn six times during the seven-week evaluation period for the RFR-exposed rats. Blood samples were not drawn during the fourth week because the biomedical technologist was ill. Weight data were taken seven times from all cannulated rats. Other activities associated with the rats involved changing the Styrofoam soil trays under all cages every other day. Food hoppers and water bottles were checked daily, with food and/or water added as necessary. On Wednesday of each week, all cages were washed using the washer described in Section IV. All water bottles were washed on Friday of each week.

C. Experimental Results

Experimental results are presented as plots showing the variations in plasma ACTH, corticosterone, and prolactin for RFR exposed and sham-exposed rats during the shakedown evaluation. Variations in growth hormone and catecholamines are not presented because, at the time blood samples were

available, the specialized equipment needed for assaying plasma catecholamines and the specialized NIH kits needed for assaying growth hormone were not available. Since then, the equipment and kits have been obtained and they are available for follow-on studies.

Variations (mean values plus standard errors of the mean) in ACTH, corticosterone, and prolactin are presented as a function of time for exposed and sham-exposed rats in Figures 9, 10, and 11, respectively.

Sham-Exposed Animals. These animals were used primarily to determine:

- the number of times the animals should be handled in order to reach the true resting level at which the lowest plasma hormone values were reached. The literature, as mentioned earlier, strongly suggests that placing rats into new cages (boxes) induces rather high stress levels. The sham-exposed animals were therefore used during this study to determine the length of the habituation period (the number of times the rats should be handled and placed in the Plexiglas boxes used for blood drawing) necessary for our animals.
- whether the resting level of plasma hormones in our animals was comparable to levels measured by other investigations. This was of special interest because the Plexiglas cages used to house our animals were of a specialized design with a bottom made of glass rods spaced 0.5 inches apart. It was realized that, while such a rod separation would be helpful in maintaining cage cleanliness, it might have forced the animals to exert additional efforts in keeping their balance.

Results of these efforts indicate that, in the case of stress hormones, the first introduction to the Plexiglas box increased the plasma level of ACTH to that representing a mild stress. Plasma levels of corticosterone and prolactin were increased more. It took two (corticosterone) or three (ACTH and prolatin) handlings of our animals before they reached true hormonal resting levels (Figures 9, 10, and 11).

Exposed Animals. During the two-week habituation period in new cages and new surroundings at the Georgia Tech RFR Facility, the rats (prior to RFR exposure) were placed twice in the Plexiglas holding boxes and left there approximately 20 minutes each time. The Week 0 data (Figures 9, 10,

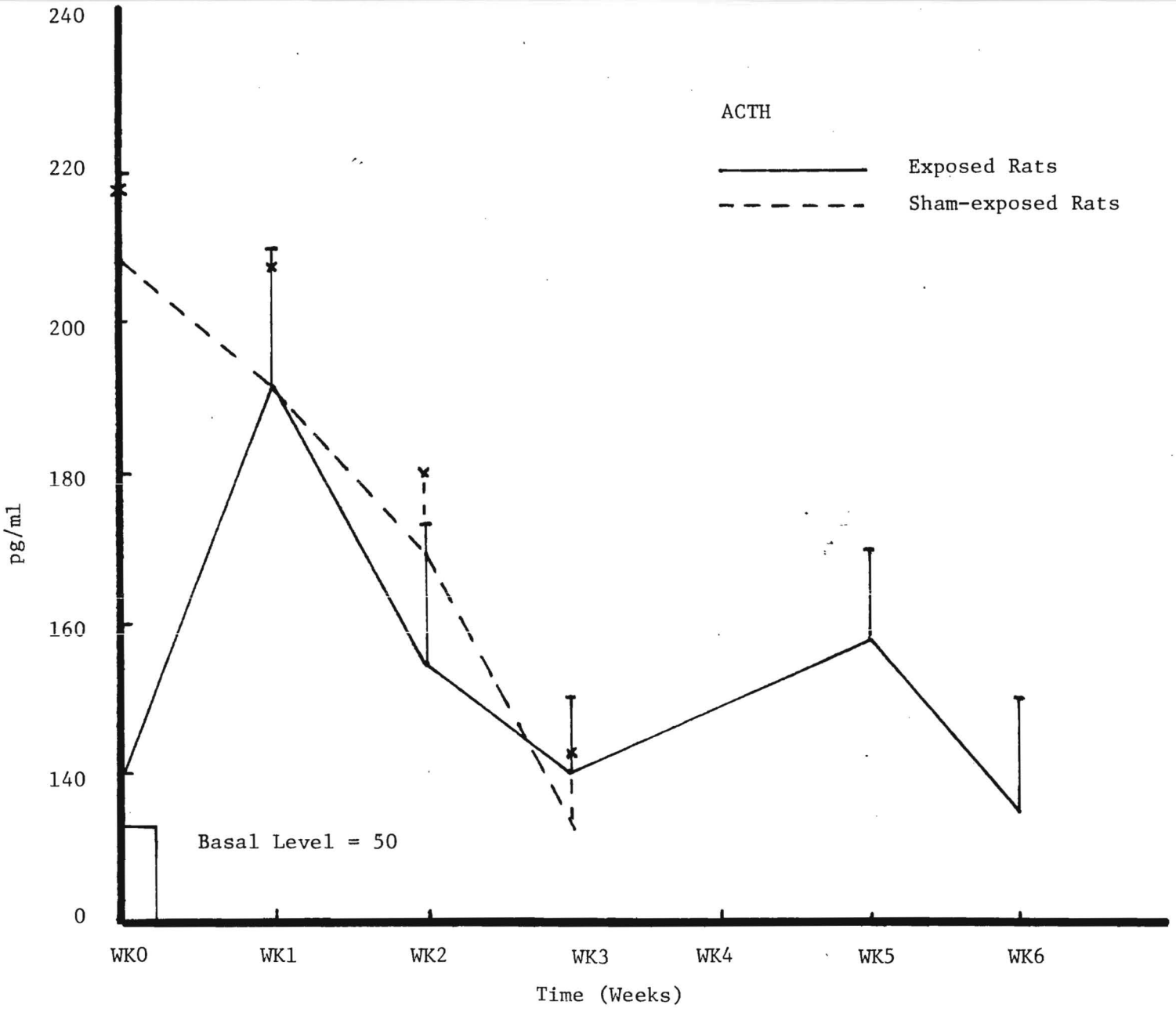


Figure 9. Variations in Plasma ACTH Levels.

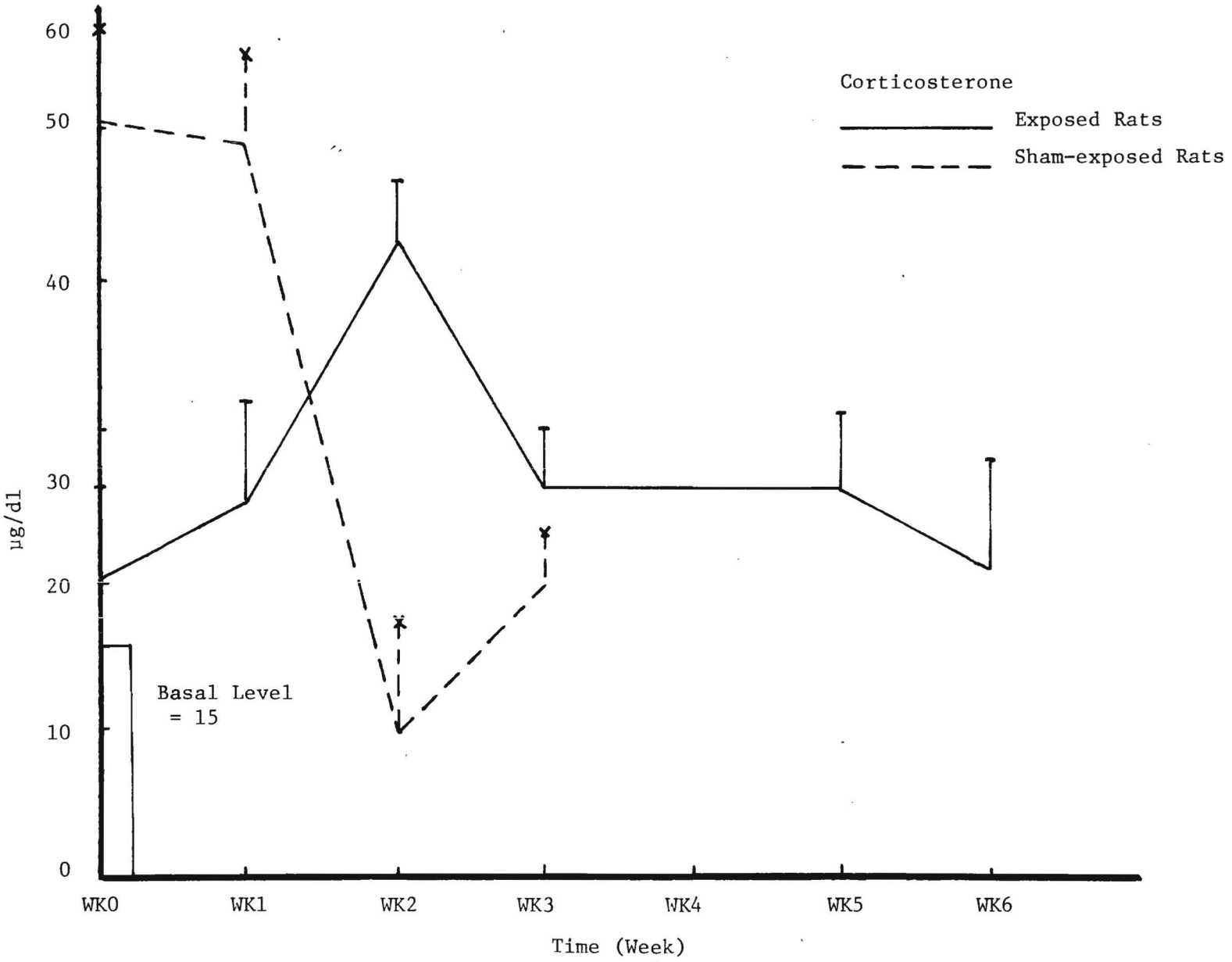


Figure 10. Variations in plasma corticosterone levels.

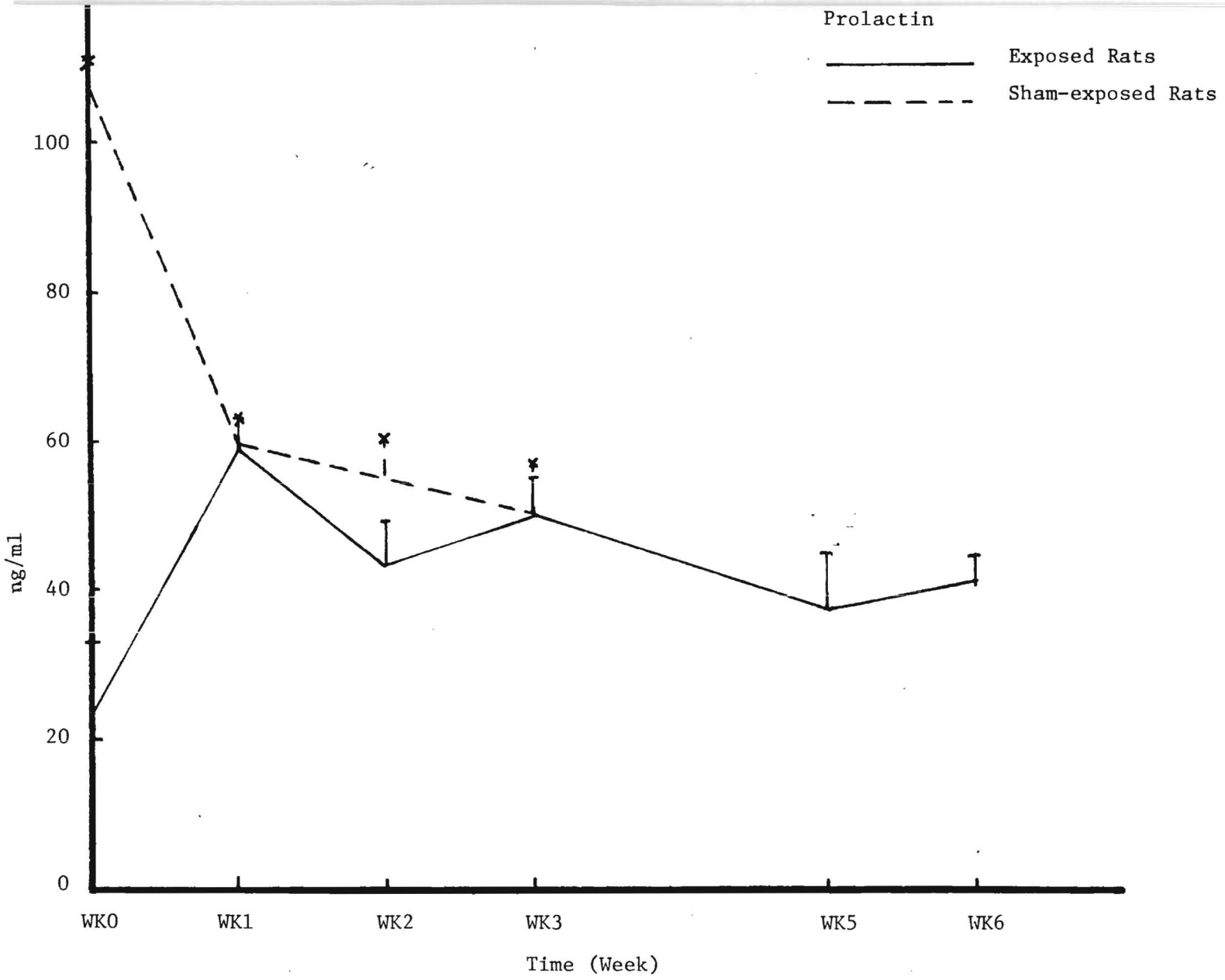


Figure 11. Variations in plasma prolactin levels.

and 11) represent the day the animals were placed for the third time in the Plexiglas boxes, blood samples were drawn for the first time, and RFR exposure was begun.

Results show an increased plasma level for all three hormones indicating an environmental stress possibly induced by RFR. The main peak for plasma ACTH occurred after one week of RFR exposure while the corticosterone peak occurred at the second week. While these two hormones returned to pre-exposure or near pre-exposure levels one week later, the plasma prolactin level remained somewhat elevated throughout the shakedown evaluation.

In summary, the results indicate that plasma ACTH, plasma corticosterone, and plasma prolactin were increased during early exposure (first and second week) to low-intensity RFR. The "stress hormones" then returned to their resting levels, suggesting adaptation to the new environment.

Results of all plasma hormone levels were available 10-to-14 days after the blood samples were drawn. This was found to be unacceptable and the flow of results between the Departments of Physiology and Pathology will be expedited to provide results within no more than four days.

Basal hormone levels as reported in the literature are also plotted in Figures 9, 10, and 11. These levels are an approximate average of a number of values presented as basal levels for the conditions under which, and techniques by which, they were measured.

During the first three weeks of the shakedown evaluation, arterial blood was drawn from an additional 25 cannulated rats and radioimmunuassays were performed. This blood was drawn on Monday of each week and two samples, rather than one, were drawn 20 minutes apart while the rats remained in the Plexiglas holding boxes. Plasma hormone levels were similar in both samples for each rats, thus demonstrating the adequacy and reproducibility of the radioimmunuassay techniques. Furthermore, the techniques of double blood withdrawal indicated that the main "stress" which elevated plasma hormones at the initial week was exposure to an unfamiliar holding box (lasting 20 minutes, or as in the case of second blood withdrawal 40 minutes) and to a lesser degree, removal from the cage. When the values of plasma hormones were decreased in Week 2 (third week of sampling), the difference between two samples was smaller, but again, the second sample did not show any decrease in values.

Weight data for the exposed and sham-exposed rats are presented in Figure 12. The sham-exposed rats entered the evaluation at a mean weight slightly less than the exposed rats. This difference was maintained throughout the evaluation without statistically significant variations as determined by the student t-test (p-values greater than 0.5 in each case). Comparisons of these data with weight data from other rats are not presented because no growth curves exist for cannulated rats housed in anything approaching the specialized Plexiglas cages used during this program.

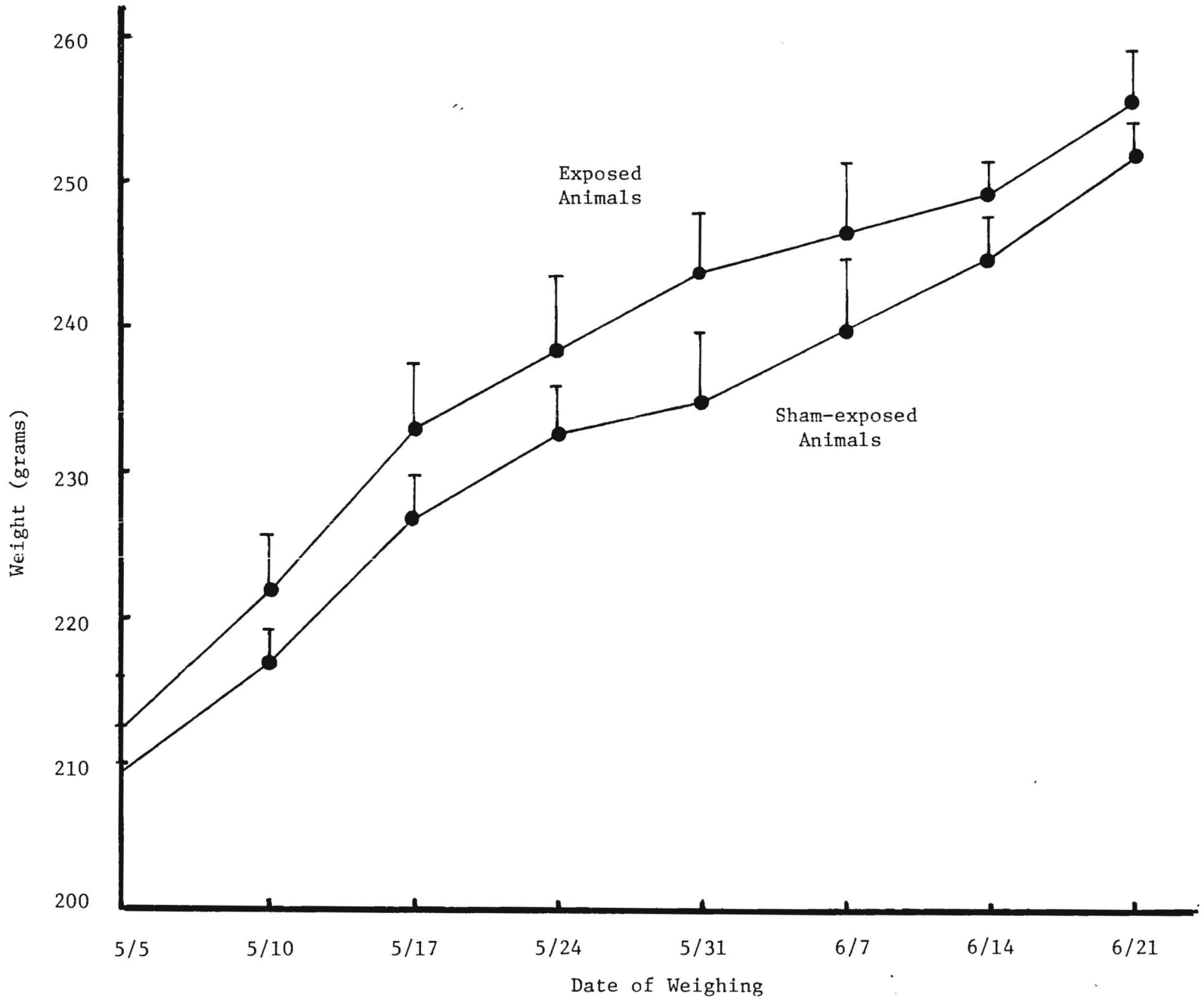


Figure 12. Weight for exposed and sham-exposed animals during the shakedown evaluation.

SECTION VIII
CRITIQUE

The primary purpose of this program was to conduct a six-week operation (shakedown evaluation) of the 435-MHz RFR Facility to assure that all tasks associated with a comprehensive long-term bioeffects study involving a large rat population could be adequately accomplished. In the proposal for the first long-term bioeffects study (Proposal No. EC-BR-1216, "Long-Term, Low-Level Bioeffects Study Using a Large Rat Population Exposed to 435-MHz RFR", submitted to AFSAM/RZP, 25 May 1982), a number of procedures based on results of the shakdown evaluation were incorporated. Now several additional modifications can be proposed as a result of new data collected after the proposal was submitted. These modifications are presented as a critique of procedures used during this program and recommended changes to these procedures.

- The specially-designed Plexiglas cages used to house rats in the Radiation and Control Rooms apparently used too few glass rods to form the floor. These rods were spaced 0.5 inches apart and possibly provided inadequate support for the rats. This resulted in a longer habituation period than would have otherwise been necessary and plasma hormone values that were above BMR levels. The number of rods in the floor has been doubled in experimental cages now being used to analyze the support provided by the cages and its influence on habituation and plasma hormone levels. It is expected that this analysis will indicate a need for improved flooring in cages used during follow-up studies.
- Persons involved in handling the rats and maintaining the Facility during this shakedown evaluation were not professionals working full-time on the program. Follow-on research programs will include a full-time person professionally trained as an animal caretaker. This will substantially improve the ability to handle large numbers of rats without inducing undue excitement. All animals will be handled by this person only, and the handling will involve use of a heavy glove placed underneath and around the animal's body.
- Both RFR-exposed and sham-exposed animals will be handled (placed in the Plexiglas holding box for 20 minutes) at least four times before initiation of the radiation.

- Microsamples of blood will be drawn from all RFR-exposed and sham-exposed animals three times before initiation of the radiation. Only after hormonal levels have fully stabilized will the actual study begin.
- Microsamples of blood will be drawn twice (day 2 and day 5) during Week 1 and Week 2 after initiation of radiation. Assay results from these blood samples will better define changes in plasma hormones during a time of possible acclimation.
- Cannulae will be sealed with a removable plastic plug (not heat sealed) that permits much longer use of the original cannulae and requires no extension parts.
- All animals will be completely undistrubed for 18 hours prior to blood sampling.
- As explained in Section VII.C, Experimental Results, the NIH kit for radioimmunoassay of rat growth hormone was not available for use during this program. The necessary kits have now been obtained with highly purified porcine growth hormone as the ^{125} I labeled antigen. Radioiodination of growth hormone will be carried out according to the methods established by D. Heber, W. Odell, H. Schedewie, and A. Wolfsen, Clin. Chem., 24:796,1978. It is noted that, for rat growth hormone assay, reagents are not commerically available. Reagents routinely used in clinical laboratories for human growth hormone determination by radioimmunoassay are not useable because of cross reactivity of rat growth hormone with corresponding human hormones.
- Active efforts are underway to implement a liquid chromatographic assay for plasma catecholamines. In the past, a radiometric technique routinely used in the clinical laboratory for assaying catecholamines in human plasma was experimental with. The technique involved several chromatographic and other steps that made it both tedious and time consuming. Further, it required 0.5 ml of plasma and it was not possible to scale it down. High performance liquid chromatography with electrochemical detection (Oka, et al., Clin. Chem., 28:646-649, 1982) offers several advantages over previous techniques (fluorometry, bioassay, calorimetry, and radioassay). This method is available for urine and brain

tissue catecholamines and will be adopted for plasma. There are indications that it can be scaled down to require only 50 µl samples.

- All assays will be performed under strict quality control rules. The same guidelines (controls, split samples, blind samples, etc.) employed in the clinical laboratory of Emory University Hospital will be applied. Participation in evaluation programs such as those sponsored by the Center for Diseased Control, the Association of Clinical Chemists, the College of American Pathologists, etc. will continue.

SECTION IX

REFERENCES

1. J. Toler, et al., "Feasibility Study to Determine Design and Construction Criteria for a 420-450 MHz Chronic RFR Exposure Facility for Rats," Georgia Tech Final Report on Project A-2228, Subcontract No. SCEEE-ARB-78-3, July 1979.

2. J. Toler, et al., "Prototype Circular Parallel Plate Facility for Chronically Exposing Large Rodent Populations to 420-450 MHz Radiofrequency Radiation, Georgia Tech Final Report on Project A-2392, Subcontract No. SCEEE-ARB-79-20, January 1980.

3. J. Toler, et al., "Construction of a 435-MHz Radiofrequency Radiation Facility for Long-Term Bioeffects Studies Involving Large Rodent Population", Georgia Tech Final Report on Project A-2650, Subcontract No. SCEEE-ARB-80-34, July 1981.

4. Antennas, J. D. Kraus, McGraw-Hill Book Company, New York, NY, 1950.

5. G. Sinclair, "The Patterns of a Slotted-Cylinder Antenna", Proceedings of the IRE, December 1948, pp. 1487-1492.

6. H. Bassen, et al., "A Miniature Broadband Electric Field Probe", Ann. NY Acad. Sci., 247:481-486, 1975.

7. V. Popovic, et al., "Technique of Permanent Cannulation of the Right Ventricle in Rats and in Ground Squirrels", Proc. Soc. Exp. Biol. Med., 113:559-602, 1963.

8. V. Popovic and P. Popovic, "Permanent Cannulation of Aorta and Vena Cava in Rats and Ground Squirrels", J. Appl. Physio., 15:727-728, 1960.

9. V. Popovic, et al., "Technique for the Introduction of Neutropenia and Granulocytosis in Rats", Exp. Hematology, 4:285-288, 1976.

10. W. B. Cannon and D. dela Paz, "Emotional Stimulation of Adrenal Secretion", Am. J. Physio., 27:64-70, 1911.

11. H. Selye, "Studies in Adaptation", Endo., 21:169-188, 1937.

12. A. W. Guy, et al., "Study of Effects of Long-Term, Long-Level RF Exposure on Rats: A Plan," Proc. IEEE, 68:92-98, 1980.

13. Z. R. Glaser and C. H. Dodge, "Review of Radiofrequency and Microwave Radiation Effects Research and Issues: 1977-1981", Abs. of BEMS 3rd Ann. Mtg., p. 31, 1981.

14. M. O. Carruba, et al., "Blood Sampling by Chronic Cannulation Technique for Reliable Measurement of Catecholamines and Other Hormones in Plasma of Consious Rats", J. Pharmacol. Methods, 5:293-303, 1981.

15. H. U. Buhler, et al., "Plasma Adrenaline, Naradrenline, and Dopamine in Man and Different Animal Species", J. Physiol. (London), 276: 311-320, 1978.

16. F. Depocas and W. A. Behrens, "Effects of Handling, Decapitation, Anesthesia, and Surgery on Plasma Noradrenaline Levels in White Rat," Cana. J. Physiol. Pharmacol., 55:212-219, 1977.

17. C. C. Chiueh and K. J. Kopin, "Hypersensitivity of Spontaneously Hypertensive Rat to Indirect Measurement of Blood Pressure", Am. J. Physiol., 234:H690-H695, 1978.

18. C. A. Blake, "Effects of Intravenous Infusion of TRH on Plasma TSH and Prolactin Concentrations in Rats", Proc. Soc. Exp. Biol. Med., 154:558-561, 1977.

19. S. W. Smith and R. R. Gala, "Influence of Restraint on Plasma Prolactin and Corticosterone in Female Rats", J. Endocr., 74:303-314, 1977.

20. G. Pinardi, et al., "Contribution of Adrenal Medulla, Spleen, and Lymph to the Plasma Levels of Dopamine-Hydroxylase and Catecholamines Induced by Hemorhagic Hypotension in Dogs," J. Pharmacol. Exp. Ther., 209:174-174, 1979.

21. B. B. Fredholm, et al., "Plasma Catecholamines, Cyclic AMP and Metabolic Substrates in Hemorrhagic Shock in the Rat", Acta Physiol. Scand., 105: 481-495, 1979.

22. S. B. Friedman, et al., "Plasma Corticosterone Response to Parameters of Electric Shock Stimulation in the Rat", Psycholsom. Med., 29:323-328, 1967.

23. M. L. Simon and R. George, "Diurnal Variations in Plasma Corticosterone and Growth Hormone as Related to Regional Variations in Norepinephrine, Dopamine, and Serotonin Content of Rat Brain", Neuroendoc., 17:125-132, 1975.

24. C. Gonzalez and T. Jolin, "Plasma and Pituitary Concentration of Growth Hormone in Male and Female Rats During a 24-Hour Period", Hor. Res., 14:130-137, 1981.

25. Y. Takahashi, et al., "Regulation of Immunoreactive Growth Hormone Secretion in Male Rats", Endoc., 88:909-917, 1971.

26. J. Seggie and G. Brown, "Coping with Stress: Parallelism Between the Effects of Septal Lesions on Growth Hormone and Corticosterone Levels", Biol. Psychi., 11, 1976.

27. C. Turpen, et al., "Stress-Induced Gonadotropin and Prolactin Secretory Patterns", Neuroendoc., 20:339-351, 1976.

28. W. P. Smotherman, et al., "Pituctary-Adrenal Responsiveness of Rat Mothers to Noxious Stimuli and Stimuli Produced by Pups", Ciba Foundation Symp., 45:5-25, 1976.

29. E. Zimmerman and V. Critchlow, "Effects of Diurnal Variation in Plasma Corticosterone Levels on Adrenocortical Response to Stress", Proc. Soc. Exp. Biol. Med., 125:658-664, 1967.

30. R. Ader and S. Friedman, "Plasma Corticosterone Response to Environment Stimulation: Effects of Duration of Stimulation and the 24-Hour Adrenocortical Rhythm", Neuroendoc., 3:378-386, 1968.

31. J. Seggie, et al., "Adrenal Stress Responses Following Septal Lesions in the Rat", Neuroendoc., 16:225, 1974b.

32. S. Eden, et al., "Plasma Levels of Growth Hormone in Female Rats of Different Ages", Acta Endoc., 88:676-690, 1978.

33. K. Takahashi, "Effects of Various Traumatic Stresses on Growth Hormone Release in Pentobarbital Anesthetized Rats", Neuroendoc., 26:1-7, 1978.

34. J. Urquhart, "Physiological Actions of Adrenocorticotrophic Hormone", in Handbook of Physiology. Endocrinology, IV, Part 2, edited by R. O. Grepp, et al., Washington, D.C., Am. Physiol. Soc. 1974, Chapter 27.

35. J. Mattheij and T. van Pijkeren, "Plasma Prolactin in Undistrubed Cannulated Male Rats: Effects of Perphenazine, Frequent Sampling, Stress, and Casturation Plus Oestrone Treatment," Acta Endoc., 84:51-61, 1977.

36. B. N. Natelson, et al., "Humoral Indices of Stress in Rats", Physiol. Behav., 26:1049-1054, 1981.

37. R. C. Kvetnansy, et al., "Effect of Handling and Forced Immobilization on Rat Plasma Levels of Epinephrine, Norepinephrine, and Dopamine- -Hydroxylas", Endoc., 103:1868-1874, 1978.

38. M. Hennessy and S. Levine, "Sensitive Pituitary-Adrenal Responsiveness to Varying Intensities of Psychological Stimulation", Physiol., Behav., 21:295-297, 1978.

39. W. F. Ganong, "The Central Nervous System and the Synthesis and Release of Adrenocorticotropic Hormone", in Advances in Neuroendoc., Univ. of IL Press, Urbana, IL, pp. 92-149, 1963.

40. H. Wakabayashi, et al., "Effect of Pentobarbital and Ether Stress on Serum Prolactin Levels", Proc. Soc. Exp. Biol. Med., 137:1181-1193, 1971.

41. C. L. Sun, et al., "Comparison of the Effects of 2-Deoxyglucose and Immobilization of Plasma Levels of Catecholamines and Corticosterone in Awake Rats", Endoc., 105(1):306-311, 1979.

42. R. Kvetnansky and L. Mikulaj, "Adrenal and Ruinary Catecholamines in Rats During Adaptation to Repeated Immobilization Stress", Endoc., 87:738-743, 1970.

43. R. Kvetnansky, et al., "Catecholamines in Individual Hypothalamic Nuclei in Stressed Rats", in Catecholamines and Stress edited by E. Usdin, Pergamon Press, Oxford, pp. 39-50, 1976.

44. R. Kvetnansky, et al., "Plasma Epinephrine and Norepinephrine Levels in Stressed Rats-Effects of Adrenalectomy", Pharmacol., 19:241, 1977.

45. R. Kvetnansky, et al., "Elevation of Advenal Tyrosine Hydroxylase and Phenyl-ethanolamine-N-Methyl Transferase by Repated Immobilization of Rats", Endoc., 87:744-749, 1970.

46. C. W. Popper, et al., "Plasma Catecholamines Concentrations in Unanesthetized Rats During Sleep, Wakefulness, Immobilization, and After Decapitation", J. Pharmacol. Exp. Ther., 202:144-148, 1977.

47. A. Ruhmann and D. Nelson, "Plasma ACTH Levels in Stressed and Non-stressed Adrenalectomized Rats", Ann. NY Acad. Sci., 297:498-508, 1977.

48. N. Yasuda, et al., "Evidence of Nycterohemeral Periodicity in Stress-Induced Pituitary-Adrenal Activation", Neuroendoc., 21:214-224, 1976.

49. R. Kvetnansky, et al., "Effects of Chronic Gaunethidine Treatment and Adrenal Medullectomy of Plasma Levels of Catecholamines and Corticosterone in Forcibly Immobilized Rats", J. Pharmocol. Exp. Ther., 209:287-291, 1979.

50. K. DeTurck and W. Vogel, "Factors Influencing Plasma Catecholamine Levels in Rats During Immobilization", Pharmacol. Biochem. Behav., 13(1):129-131, 1980.

51. J. D. Dunn, et al, "Daily Variation in Rat Growth Hormone Concentrations and the Effect of Stress on Periodicity", Neuroendoc., 13:69, 1974.

52. G. Brown and S. Reichlin, "Psychologic and Neural Regulation of Growth Hormone Secretion", Psychosomat. Med., 34:45, 1972.

53. J. Seggie and G. Brown, "Stress Response Patterns of Plasma Corticosterone, Prolactin, and Growth Hormone in the Rat Following Handling or Exposure to Novel Environment", Can. J. Physiol. Pharmacol., 53:629, 1975(a).

54. R. Collu, et. al., "Diurnal Variations of Plasma Growth Hormone and Brain Monoamines in Adult Male Rats", Can. J. Physiol. Pharmacol., 51:890,1973.

55. J. Martin, et. al., "Pulsatile Growth Hormone Secretion: Suppression of Hypothalamic Ventromedial Lesions by Long Acting Somatostatin", Science, 186:538, 1974.

56. B. Natelson, et al., "Relationship Between Avoidance-Induced Arousal and Plasma DBH, Glucose, and Renin Activity", Physiol. Behav., 18:671-677, 1977.

57. R. A. Assaykeen, et al., "The Sympathetic Nervous System and Renin Secretion", in Frontiers of Neuroendoc., edited by L. Martini and W. F. Ganong, Oxford University Press, NY, pp. 67-102, 1971.